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PHYSIOLOGICAL REVIEWS

VOL. VI JANUARY, 1926 No. 1

RED AND WHITE MUSCLE

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That skeletal muscle may vary in colour, not only from species to species, but in the muscles of the same animal, has long been known. The difference between the whiteness of fish muscle, and the deep crimson of the pigeon's breast is striking indeed; in the rabbit all shades are found between greyish-cream and red, many individual muscles being shaded in colour, appearing redder the deeper one penetrates toward the bone.

Many early workers, for example Boerhaave (8) in the eighteenth century, believed that the red colour was due to a greater supply of blood, and there was much discussion on the point before any experimental work was done. But in 1865, Kühne (41) described experiments in which he washed out the vascular system of rabbits with saline solutions; he then prepared extracts of the entirely blood-free muscle by mincing finely and mixing with an equal weight of cold water. The extract from the pale muscle was colourless, that from the dark muscle was red. He examined the absorption-spectra of the red extract, and of thin strips of red muscle, and came to the conclusion that the pigment present was hemoglobin. This he confirmed by preparing derivatives—reduced hemoglobin, hematin, COhemoglobin and reduced hematin. On microscopic examination, he saw the red pigment, not in granules, but apparently in solution in the fibrillar substance, between the doubly-refracting sarcous elements.

About five years later, Ray Lankester (43) suggested that a red pigmentation is found in muscles from which the most persistent and prolonged activity is required. A general survey of the animal kingdom bears out this view; and in the lower animals, nearly all the muscles (except the constantly acting heart which is always pigmented) are colourless; but in mammals all, with few exceptions, are red. Lank-

ester particularly studied the gastropods. In molluscs the pharynx muscle, which is the strongest and most active in the body, is red; and on spectroscopic examination of the pigment from Limnaeus and Paludina, he found that it was identical with hemoglobin. This case of the molluscs is especially interesting, as the blood of these forms is colourless, and the presence of pigment in their muscle is thus a powerful argument against the view held by Brozeit (11) and others about this time, that the muscle colour, if not due to circulating blood, might yet be due to broken-down corpuscles.

Ranvier in 1873 (63) was the first to bring definite experimental evidence of the different behaviour of the two types of muscle. He showed that more stimulations per second were needed to produce a smooth continuous contraction with white muscle than with red. In other words, the twitch movement is a slower process in the red muscle than in the white. He was able to obtain curves with Marey's instrument to show this for the rabbit and also for the ray fish (which, like most fish, has a series of small red muscular bundles just under the skin in the dorsal area). Ranvier supported Ray Lankester's theory as to the functional differences of the muscles, and carried the histological studies further.

These investigators thus opened up the three fields of histology, physiology and spectroscopy, approaching each with a wide and comparative outlook. For the next thirty-five years these fields were the scene of much activity on the part of many different workers. But now they are almost deserted; since 1909 attention has been almost exclusively directed to the chemistry of the two kinds of muscle in attempts to discover the chemical differences, both static and dynamic, which must underlie the functional differentiation.

Histology. In 1875 (46) Meyer examined the adductor and semitendinosus muscles of the rabbit, and confirmed Ranvier's findings. But he discovered also that other red muscles, e.g., the flexor digitorum communis and the masseter, agreed in structure rather with the white adductor than with the red semitendinosus. He came to the conclusion that the structure of the semitendinosus was specialised and at first thought that this might be due to some relationship, characteristic of rodents, between the semitendinosus and adductor groups. But on examination of the muscles of the hare, squirrel, mouse, rat and guinea pig, only in the last did he find a state of affairs similar to that existing in the rabbit. From this he concluded that the marked differentiation in the muscles of the rabbit and the guinea pig was a result of domestica-

tion. He regarded the white muscle as abnormal or due to disuse; the red muscles with the masseteric structure, as normal; and the structure of the red semitendinosus as pathological because of the almost continuous contraction (unusual for a flexor) in the domestic rabbit.

Ranvier (63), undismayed by these contradictions, showed later in support of his views that in the wild Garenne rabbit the same differences were present which he had noted in the tame variety.

Grützner (30) began to study the question of red and white muscle about 1883; his work is best considered in the physiological section, but it may here be said that, partly from histological studies, and partly from the results of experiments showing a different type of response according to the intensity of the stimulus, he came to the conclusion that every muscle contains two types of fiber, often intimately mixed, one kind fine and dark in colour, that other larger in cross-section and bright in appearance. The dark colour of the fine fibers is probably due to the presence of numerous granules in the sarcoplasm between the fibrillae. Grützner believed that all the fine granular fibers, whether pigmented or not, corresponded to the red, slowly-contracting muscles of the rabbit; and that the larger, clear fibers corresponded to the more rapidly moving white muscle.

Rollett (66) and Knoll (38) however, both, after several years of work, concluded that that statement was too sweeping. Rollett published many elaborate studies of the histological appearance of striated muscles of various animals, particularly of flies, and of certain mammals such as the field-mouse. He pointed out that though difference in function is often associated with difference of structure, yet these latter differences may not always be found, and when present, structural variations cannot be interpreted as pointing to functional variations in the absence of other evidence. In the case of the field-mouse, the muscles behave as slow muscles and have the structural characteristics of such muscle. But with the flies, Dytiscus and Hydrophilus, the leg muscles of the former contract rapidly and of the latter slowly, though all are uncoloured. Moreover, the nuclei in Dytiscus have the arrangement usually characteristic of red muscle, being more numerous and more deeply arranged, even within the striated muscle-substance. Knoll was particularly interested in the protoplasm-like substance between the fibrils; indeed, he refers always, not to red and pale fibers, but to "protoplasma-armen" and "protoplasma reichen" fibers. In papers published from 1889 to 1891 he goes through the different classes of the animal kingdom noting the occurrence of deeply pigmented muscles,

and of muscles differing notably in activity, and examining the histological structure of these, with special reference to the amount of sarcoplasm and the number and kind of granules in it. In the crab, the heart-muscle is brownish-yellow, with granular sarcoplasm, in contrast to the rest of the musculature. Then the thorax muscles of the Diptera, Neuroptera, Lepidoptera and flying Coleoptera are reddish-yellow and granular while the rest of their muscles are unpigmented and in most parts clear. The closing muscles of the Lamellibranchiates are neither red nor granular, and in most Gastropods the pharynx muscle, unlike the body-muscle, is purplish-red, with small granules between the fibrils. In the Cephalopods the heart is very granular; in the fishes certain muscles are red and granular, especially just under the skin; also the heart—and in some fishes such as Hippocampus especially the fins. In the frog, all the muscles except the heart and superficial neck muscles, are white; but mixed with the characteristic large clear fibers are more or less numerous fine granular fibers. Amongst the birds, both red, granular and white clear muscles are to be found, while mammalian muscle is mixed. In a few mammals, for example, the rabbit and the guinea pig, the red and pale muscles are separated, but the pigmentation and degree of granulation do not always run parallel. The general conclusion is that in the lower animals the red and granular formation is confined to the most important muscles, while in the higher animals, only the least active muscles are white. For instance, among the birds, the domestic hen and the pheasant, which fly little, have white breasts and pigmented leg muscles, whilst the pigeon, falcon and starling, which are strong in flight, have deep red breast muscle and somewhat paler leg muscle.

As regards the nature of the granules, Knoll found that some would stain with Flemming's solution and so probably consisted of fat, while some did not blacken in this way, but gave an intense red with gold chloride. From this and other reactions he came to the conclusion that a lipoid substance of some sort was concerned. He thought also that he could see granules of an intermediate character, and that conversion of the one into the other could take place.

It thus appears from the work of Rollett and Knoll that although in many cases slow, sustained contraction is found associated with hemoglobin-containing, slender, granular, characteristically-nucleated fibers; yet these attributes do not always go together. Pale muscles may be as granular as dark ones, and they may be no larger in cross-section; the arrangement of the nuclei varies in both types; muscles of the same

colour may show different rates of movement, while in a few cases the red may move more rapidly than the white.

In 1890, Grützner's pupil, Bonhöffer (9) published a study of the histology of frog muscle. Krause had suggested that the different kinds of fiber seen by Grützner in the leg muscles of the frog were not different types of muscle at all, but the same kind of muscle at different ages—the thin granular kind being the younger. Bonhöffer, by examining the sections of the whole leg from animals of various ages, was able to prove that the proportion and distribution in space of the two kinds of muscle was the same whatever the age of the frog. The gastrocnemius always contained chiefly dark fibers, and the sartorius clear ones. Schaffer (69) also studied the different appearances under the microscope, of the two types of muscle, particularly from man, (where they are found intimately mixed), paying special attention to the arrangement of the fibrils in the sarcoplasm. He reported that on the whole the clear sarcoplasm contained bundles of fibrils small and regularly arranged, whereas, in granular sarcoplasm, the larger fibrils were grouped quite irregularly. He drew attention to some pitfalls in histological interpretation; especially that brightness of fibrils cannot always be taken as an index of real morphological difference. Fibers fixed while contracting or degenerating appear brighter than normal.

Ten years later, Paukul (57) in 1904, determined the form of the twitch and the histological structure of twenty-one different muscles in the rabbit. He concluded that the slow muscles were always red, but the red muscles were not always slow. In particular the masseter was red, but contracted rapidly—this fact might possibly be explained by the strength and continuity of its activity in rodents. He confirmed Schaffer as to the distribution of fibrils in the sarcoplasm.

Physiology. After Ranvier's beginnings, the physiological work was next carried on by Kronecker and Stirling (40) who published a paper in 1878. They confirmed Ranvier's result that a greater frequency of stimulus is required to produce tetanus in white muscle than in red, but using improved apparatus, they obtained smaller figures—10 stimuli per second for the red, and 20 for the white, where Ranvier had given 55 and 357 respectively. In the twitch, greater height of contraction is obtained with white muscle, but in incomplete tetanus the simple contractions are superimposed on the ascending part of the curve, and the curve for red muscle rises considerably higher than that for white.

Cash (13) investigated the gracilis, gastrocnemius, triceps and semi-

membranosus of the frog and the toad; he found that the gastrocnemius was the slowest of these (compare the histological work of Bonhöffer, mentioned above) and that the quickest muscle of the toad was slower than the slowest of the frog.

Adducco (1), Rollett (66) and Grützner (31) all about the same time (1886), and Paukul (57) much later performed experiments independently upon various animals—the rabbit, field mouse, insects, frog, etc.—along the same lines. The result was the proof that in nearly all cases the white muscle gives a higher twitch than the red (often three to four times greater), but the red muscle contracts more in tetanus than does white and can maintain itself in that condition for a longer time. Some of the most recent work is contained in a paper by Fischer (26) published in 1908. In a careful study of the soleus (red) and the gastrocnemius (pale) of the cat, he came to the following conclusions. In the first place there was no difference in latent period. Secondly, the twitch of the soleus was more prolonged and (strangely enough) slightly higher than that of the gastrocnemius. Thirdly, the tetanus of the white is much less marked than that of the red muscle and the maximum shortening is only obtained with much greater frequency of stimulus. Fourthly, the tension curve for the isometric twitch is prolonged for the soleus, but lower than that for the gastrocnemius, in spite of the fact that the actual contraction is somewhat greater. In the fifth place, the tension is much longer maintained in the tetanus of the red than in that of the white. In its rapid twitch the latter reaches a tension of 600 grams, and in tetanus 700 grams, for a very short time; with the soleus, the tension of the twitch is only 200 grams, but if the stimuli are repeated, it can maintain a tetanus of 900 grams for a long period. The muscles of the rat, guinea pig and rabbit gave similar results, save that with them the soleus twitch was never so great as that of the gastrocnemius.[1] In a paper in Brain in 1916, Roberts (64a) gives a discussion of the function of red muscle, analysing the probable functions in various animals—for example, the falcon, skate and rabbit. He points out that in birds like the falcon, the proximal segments of the wing remain permanently extended by prolonged contractions of the pectoral muscles, while the distal segments perform the necessary rapid movements. So that the pectoral muscles

[1] Riesser (58b) in 1921 described a method for keeping excised mammalian muscles under the best conditions for survival. He gives a detailed description of the twitches of red and white muscle, paying special attention to the meaning of the "Funkeschen Nase" on the curve for the red muscle.

are characterized by slow movement as well as by great endurance. Consideration of the three chief red muscles in the rabbit—the soleus, crureus and the deep head of the triceps—leads to the same conclusion. Further, white muscles often pass over two or more joints, while the more deeply seated red muscles usually unite adjacent segments of a limb. A white muscle may cause movement at either of the joints over which it passes, but clearly it can only exert the full effect on one joint if the other is held firm. Such fixation may be a function of red muscle.

We must now consider the various theories that have been put forward to describe this connection between structure and function. The earliest was that of Grützner (31). As we have seen, he believed that all muscles contained a mixture of two types of fibers, corresponding to the red and white of the rabbit. According to his view, the response of a muscle on stimulation depended on the proportions in which the two types were present. There are many puzzling points which Grützner was able to explain on this theory. For example, a muscle was stimulated with weak induction shocks repeatedly, and the indented curve of an incomplete tetanus obtained; if now the shocks, though timed as before, were increased gradually in strength, the degree of contraction of the muscle increased and the tetanus became complete. Grützner's explanation of this was that the irritable white fibers were first stimulated, then, with the stronger shocks, the less irritable red fibers began to respond with their slower movement and greater capacity for contraction, eventually causing the twitches of the white ones to become invisible on the curve. Again, under the proper conditions (with the muscle sunk in saline and quite unloaded) it is possible to show that the response to the weak stimulus is quite different in quality and direction to that resulting from a strong stimulus. von Kries (39) had shown that, if a muscle is stimulated by two or more weak shocks, the rise of the second twitch is more rapid than that of the first, and von Frey (28) had demonstrated a similar more rapid and higher rise with a single stimulus and a supported muscle. Grützner combined these two observations by suggesting that in von Kries' case the support is afforded by some part of the muscle itself. The first stimulus had excited the red fibers, and being slow to relax, they would be still in contraction at the time of the second stimulus, thus raising the second response. Tigerstedt (70) agreed with the theory of Grützner. By applying non-polarisable electrodes to different parts of the frog gastrocnemius, he was able to obtain curves, some corresponding almost entirely with those for red muscle, and some with those for white.

Bottazzi's theory (10) was advanced some ten years later, in 1897. He had been studying the "oscillations of tonus" in the auricles of the tortoise heart. This muscle is very rich in sarcoplasm, which Bottazzi supposed resembled the undifferentiated protoplasm of amoebae; like the latter, the muscle showed a very slow rhythm of contraction, and was particularly sensitive to mechanical and thermal stimuli. The tracing for the auricular movements is extremely interesting. It shows large, slow contractions, on which the heartbeats are superimposed; the striking thing is that when the tonic contraction is at its height, the heart-beats show the greatest amplitude, while as the tonic state falls off, they diminish accordingly. Bottazzi suggested that the sarcoplasm was responsible for the large and slow variations in tonus, while the striated portions of the fibers brought about the more rapid beat. He imagined that the sarcoplasm was playing the part of the "intrinsic support" of Grützner. As the red muscles are richer in sarcoplasm, they would, on this theory, as on Grützner's, play the chief rôle in providing the support.

Mosso (50), in 1904, criticised both these theories. He considered that that of Grützner was not sufficiently proven, and as for Bottazzi's, though it might fit the facts for the lower animals, it certainly could not apply to the muscles of man and the higher animals, where the sarcoplasm is very scanty but the capacity for tonus very high. He drew attention to the discovery, shortly before claimed by Perroncito (68) of the double innervation of striated muscle, and suggested that the variations in tone might be brought about by stimulation of the sympathetic fibers, while quick movements might depend on stimulation of the motor nerve. As a matter of fact, it is now known that Perroncito misinterpreted the histological appearances, the so-called sympathetic fibers being unmyelinated collaterals. In 1913, however, Boeke (7) and Agduhr (2a), who extended his work, were able to show quite definitely that grey rami communicantes (arising in the thoracio-lumbar ganglia of the sympathetic cord) do pass to striated muscle, and they were of the opinion that both kinds of nerve endings are found in the same muscle fiber. But Kulchitsky (41a) confirmed by Hunter and Latham (34) could find no case of the same fiber receiving both sympathetic and somatic nerve endings. J. I. Hunter (36a) in a lecture to have been delivered in London, published posthumously this year, supported the view that the skeletal muscle of vertebrates consists of two sets of muscle fibers, the slender granular type chiefly concerned with maintenance of posture, and the larger, clearer type with move-

ment. He believed that only the former received sympathetic innervation, while the latter were supplied only with somatic nerves, and he was able to bring foward much evidence (36b). It is well known that section of the nerve to a muscle causes decrease in size of the muscle fibers by as much as 60 per cent. Hunter and Royle (34a) showed that if the lumbar sympathetic chain was excised, *or* the somatic innervation removed, only *some* of the muscle fibers, and those in groups, became smaller. This grouped arrangement of the two types was confirmed by observations of Latham (44a) who found that fibers receiving non-medullated nerves are often situated in groups. Hunter and Royle (34a) found that, in decerebrate rigidity, sympathetic denervation led to a marked alteration in tone: the condition of plastic tone is abolished, so that, although the animal still becomes rigid in the characteristic extended attitude, it no longer shows the power of maintaining a limb in any position artificially imposed upon it. Thus the authors suggested that plastic tone is maintained by one type of muscle fiber under sympathetic control, and contractile tone by the other type of fiber under somatic control. These experiments were chiefly performed on goats, but operations on patients suffering from spastic paralysis, where sympathetic denervation brought relief, show that a similar state of affairs exists in man.

A very large amount of work has been done on the effect on the tonus of skeletal muscle of stimulation of the sympathetic nervous system, whether electrically or by the use of drugs, but in most cases any differentiation of muscle fibers in the muscles used has not been considered. The important work of Riesser and of Neuschlosz is referred to later.

Studies on the pigment. Investigators since Kühne who have studied the absorption-spectra of the muscle-pigments, seem to have come to the conclusion that the latter, although closely resembling hemoglobin, is not identical with it. MacMunn (45) described a number of tissue pigments, which he called the histo-hematins, and he claimed that they were not identical with any known breakdown-product of hemoglobin. The pigment found in the muscles he named myohematin, and described its bands as always present in three places in its spectrum (613–593, 569–563, 557–548). Mörner (49) also believed that the pigment, which he called myochrom was different from hemoglobin, but his description differs from that of MacMunn. In more recent times (1921), Gunther (32) has renewed this study; he concluded that the muscle-pigment, though resembling hemoglobin so nearly that it gives all its characteristic chemical reactions, yet cannot be identical with it, because the maxima of the absorption-bands are rather different,

as well as the spectra of the derivatives. He speaks of myoglobin, metamyoglobin, myohematoporphyrin, etc.[2]

The possible significance of this muscle-pigment must now be considered, for it can hardly be a coincidence that it is so generally associated with a particular type of muscle. It is odd that in the work on spectroscopy, so much attention has been paid to the comparatively unimportant question as to whether or not the pigment is identical with hemoglobin, while the problem as to whether the pigment, whatever it is, can perform any function as an oxygen-carrier, has been left almost untouched. No such work seems to have been attempted with myoglobin as was tried by Hentze (33) for hemocyanin. Perhaps this is because myoglobin has never been isolated. What is needed, and what could be now attained, is a determination with modern gasometric methods of the oxygen-combining power of the extract. Gunther believes that myoglobin is probably a respiratory pigment, but his opinion is only based on the researches of MacMunn 35 years before, and of Kühne, 20 years earlier still.

The evidence from Kühne's work for a respiratory function of the pigment is based on the following experiments. He examined the absorption spectra of the thin muscles of the toad; some of them were resting and others had just been active. He found the latter of a darker colour than the former, and he found that upon oxygenation this darker colour could be replaced by a ligher one. Such work is not very convincing and is full of difficulties; for example, it was impossible to wash out the blood vessels of the animal so as to exclude the possibility of the presence of hemoglobin, and it was impossible to use an animal whose vessels could be perfused, because mammalian muscle, such as that of rabbit and hare, so readily loses its irritability. The only evidence which can be adduced from MacMunn's work is that the pigment can be alternately oxidised and reduced by dipping the tissue into ammonium sulphide and then exposing it to air.[3]

[2] A very recent paper by Keilin (80) goes far towards clearing up this confusion. He finds that two distinct pigments occur in muscle: (1) a hemoglobin (= myoglobin of Gunther, myochrom of Mörner) which, though not identical with the blood hemoglobin of the same animal, differs from it no more than the blood hemoglobin of one animal differs from the blood hemoglobin of another; (2) cytochrome (= myohematin or histohematin of MacMunn) of which the composition is still uncertain, but which may consist of three hemochromogens. Some muscles, e.g., the frog's heart and legs, contain only cytochrome; some, e.g., the pigeon's breast, contain both pigments.

[3] See Keilin's paper (loc. cit.) for alternate oxidation and reduction of cytochrome in vivo in the thoracic muscles of the wax-moth.

One view of the meaning of the pigmentation, of course, is that it is to assist oxidation during the prolonged contraction of the muscle. In this connection the work of Robinson in 1924 (65) must not be forgotten. She was able to show that all the iron-containing derivatives of hemoglobin can function as catalysts of certain oxidative processes, quite apart from any rôle of oxygen-carrier.

An alternative view, and one perhaps equally supported by the facts, is that the myoglobin is a breakdown-product of protein metabolism which cannot be further destroyed. Needham (52) has recently pointed out that the distribution of the histo-hematins is in accordance with this theory, that they were end-products of metabolism, and that the occurrence of myohematin in active muscles might just as well be a result of that greater activity as a provision for assistance. As we shall see later in the discussion of tonus, it is just in muscles of the slow moving type that protein katabolism may have a special importance.

Gunther (32) studied the ontogeny of the pigmentation. He found that the muscle of embryo rabbits is practically all of one colour. In the human embryo, up to the third month, there is practically no coloration, even in the heart-muscle; from the third to the seventh month there is a very slight increase, and after this no further change till the muscle becomes active. The present writer has observed a marked difference between the pale yellowish leg muscles of the embryonic chick at the nineteenth day, and the reddish leg muscle of the chick three days after hatching out. It was stated by Nasso (51) in 1879, that the pale muscle of the calf changes to the darker colour of the adult when the diet is changed from milk to green food, but this has never been confirmed.

Chemistry. It is interesting to note that before 1909 there were only two isolated attempts in the literature to discover any chemical difference between the two types of muscle, while since that date papers on the subject have come thick and fast, especially since the War, from the Frankfurt school.

Gleiss' paper in 1887 (29) deserves special mention because not only is it unique for its time as regards the problem tackled, but it approached the matter in a distinctly modern fashion, asking from a dynamic standpoint what manner of chemical changes take place in the two kinds of muscle; not merely what their constituents might be. Gleiss found that upon activity or rigor mortis, the red muscle becomes much more acid than the white. He pounded up the muscles in salt solution, containing litmus, and compared the muscles of the toad and the frog,

and the red and white muscles of guinea pig, rat and cat. By testing with ferric chloride he came to the conclusion that the acid produced was lactic acid. One paragraph at the end of his paper is well worthy of quotation: "Es scheint mir also aus allen diesen Versuchen hervorzugehen, dass der langsamer arbeitende rothe Muskel auch sparsamer arbeitet und weniger Zersetzungs-produkte liefert als der weisse. Da mit der Bildung der Zersetzungs-produkte die Wärmebildung Hand in Hand geht, dürfte dasselbe von letzterer wohl auch als wahrscheinlich angenommen werden." Although of course, the notion of the food as the fuel of the body had been familiar since the days of Lavoisier, and especially since the work of Liebig and Voit, yet the idea of a particular chemical reaction being responsible for the energy-production in a definite tissue was an original one for the time.

After this, the introduction to Knoll's histological paper in 1891 (38) is disappointing reading. After saying that he cannot believe that the functional significance of histological differences can be solved merely by the study of twitch curves, because there may well be functional differences not expressed on those curves, he continues: "It appears to me still less profitable, in the uncertainty of our knowledge of the functional meaning of the individual chemical substances contained in striated muscle, to attempt to answer this question through the study of chemical differences between red and white muscle in states of rest and activity."

In how different a position are we now for an attack on the problem? Amongst the substances which have been investigated in red and white muscle, whether as to quantity present or as to metabolic relationships, may be mentioned lactic acid, phosphoric acid, inorganic salts, purine bases, creatin, carnosine, cholesterol, succinic acid and myosin. Of these at least lactic and phosphoric acids have a definite functional importance in muscular activity, and we can form theories with some degree of probability as to the rôles of cholesterol, succinic acid, myosin, creatin, potassium, calcium.

One of the most important comparisons of the chemistry of red and white muscle is contained in the paper of Fletcher (27) in 1914 on lactic acid formation in mammalian muscle. He found that in excised muscle kept at room temperature, lactic acid formation goes on much more quickly in the white muscle than in the red and the maximum reached in the white is higher. For example, he obtained the following figures:

	AFTER 3.25 HOURS	AFTER 5.75 HOURS
Red	0.219 gram % ZnL	0.327 gram % ZnL
White	0.438	0.466

Now from the work of Meyerhof (47) it is proved that, at any rate in the frog, the breakdown of carbohydrate into lactic acid and the combustion of part of the lactic acid so formed, is the source of the energy of muscular movement; moreover, the production of hydrogen ions is probably responsible for the actual mechanism of the motion. When we consider this, we realise how important for any understanding of the different types of activity in the two muscles is this work of Fletcher's. His result has been confirmed for rabbit muscle by the present writer in 1924 (50b) who has been able to show that the same kind of thing happens with the hen, where the lactic acid maximum is for the leg muscle only just over half that of the muscle from the breast. In the case of the pigeon she found that just the reverse happens; the value for the light red muscles is approximately the same as for the pale muscles of the hen, but that for the deep red pectoralis major is abnormally high, even up to twice this amount. We shall see later that this can be correlated with the work of Lyding (44) on phosphoric acid metabolism.

We must now go back a little, to 1909, to study the question of creatin. This matter has never really been attacked from the point of view of explaining the difference between red and white muscle, but of elucidating the characteristic behaviour of any muscle in tonus. Now tonus is a state of prolonged contraction, accompanied by very little increase in metabolism or at any rate of carbohydrate metabolism, and unlike tetanus, not associated with large variations in electrical response; it is the normal method of contraction in unstriated muscle and a very striking example of it is to be found in the adductor muscles of the bivalve, Pecten. It is clear that certain resemblances may be traced between the contraction of unstriated muscle and of red muscle; indeed the contraction of red muscle may be described as being more tonic than that of white. On deeper consideration we find that all voluntary muscle may exhibit some degree of tonus, and usually does, except when quite flaccid as during sleep, after section of all nerves, or in certain pathological states such as Amyotonia congenita. Now Pekelharing and van Hoogenhuyze (59) suggested that tonus, if not associated with carbohydrate metabolism, might be associated with the metabolism

of nitrogenous bodies; and in support of this they described experiments in which they had found more creatin than the normal in muscles of animals in a state of decerebrate rigidity and in muscles in contracture after various poisons. They also found very little creatin present in muscles which had been denervated some time previously, and ascertained that ordinary contraction made no difference in the creatin-content of the muscles of the frog. Riesser (58a) obtained results which at first seemed to confirm this theory. He found that poisoning with certain drugs, e.g., tetra hydro β-naphthylamine, which are believed to stimulate the sympathetic nervous system, or subjection to cold (which may have the same action) caused increased creatin content in the muscle. In view of the theory that increase in tone depended on the activity of the sympathetic nerves, it was generally assumed that the creatin formation was associated with the increased tonus. But Pekelharing and van Hoogenhuyze had already observed a fact somewhat opposed to this view—that red muscle contains less creatin than white muscle; and Riesser also pointed out that smooth muscle, most remarkable for tone, contains less creatin still. The following are some of the figures found or quoted by Riesser:

	WHITE	RED	HEART MUSCLE	SMOOTH MUSCLE
	gram %	*gram %*	*gram %*	*gram %*
Hen	0.34	0.22	0.15 0.08	
Rabbit	0.41–0.50	0.22–0.32	0.12–0.24	0.02–0.09

Furthermore, Riesser working with Hamann (58) has failed to confirm the creatin increase found by Pekelharing and van Hoogenhuyze with various poisons, and also the increase they found on heat and death rigor. He remarks, however, that much of this work is really beside the point, as obviously heat and death rigor, and almost certainly all the contractures investigated (except that with nicotine) have nothing in common with physiological tonus. The matter thus remains in a state of uncertainty. An investigation of the effect of contraction on the creatin content of red muscle does not seem to have been carried out; it is just possible that although the resting and post-mortem values are lower than for white muscle, an increase might take place under these conditions where no increase occurs in white.

Another nitrogenous tissue-constituent which has been investigated

in red and white muscle is carnosine, which is interesting because it is the only known dipeptide besides glutathione in the tissues, and the only instance of a β-amino acid occurring in the body. Unfortunately, the results obtained by Clifford (14), Hunter (36) and Mitsuda (48), are so contradictory that little can be deduced from them at the present time.

Nor does one know how to interpret the results of Rinaldi (64) for the purine bases; he found that the white muscle contained slightly more than the red, but that they formed a slightly higher percentage of the total nitrogen in the latter type of muscle.

As long ago as 1882, Danileffski and Schipiloff (19) showed that if all the protein known as myosin is extracted from a muscle by dilute salt solution, a locular structure remains which is almost isotropic.

TABLE 1

ANIMAL USED	MYOSIN : "GERÜSTSUBSTANZ"
Ox (red)	1 : 1.5
Calf (white, but growing)	1 : 4.4
Frog (white)	1 : 0.8
Toad (red)	1 : 1.5
Dove, breast (red)	1 : 5.6
Dove, leg (white)	1 : 1.1
Hen, breast (white)	1 : 3.1
Hen, leg, (red)	1 : 0.86
Starling, breast (red)	1 : 4.5
Starling, leg (white)	1 : 0.94

They concluded, therefore, that the anisotropic substance which normally filled up the spaces consisted mainly of myosin. They investigated various kinds of muscle, comparing the proportion of myosin to "gerüstsubstanz" in each and suggested that the quicker a muscle contracted and relaxed, the more gerüstsubstanz it had. This is of course exactly contrary to what we should expect on histological grounds and, indeed, their figures do not bear out their theory. Their mistake seems to have been that they usually compared one species of animal with another, whereas exact results can only be arrived at by comparing different muscles in one and the same animal or the same muscles in very nearly allied animals such as the frog and the toad. When this can be done with the figures of Danileffski and Schipiloff, we see that the results do fit in with the histological evidence, except for the hen and the case of the ox and the calf. It is probable that the latter cannot

really be counted as an exception, for the larger proportion of "gerüstsubstanz" in the white muscle is probably connected with the growing state of the animal, the muscle cells not yet being completely differentiated into fibrillae (and cf. the results of Hotta (35) which will be referred to later). Danileffski and Schipiloff's figures are summarized in table 1.

Quagliarello (60) in 1914 only confirmed these early workers when he showed that the juice from white muscle (containing numerous doubly-refracting granules, which he took to be the debris of the fibrillae) contains more myosin than that from the red. In a later paper (61) he found that the total nitrogen and the extractive nitrogen were greater in the white muscle of birds and rabbits.

It has been recognized for some years that the distribution of phosphorus between the organic and inorganic fractions in muscle tissue is a matter of great interest. In 1915, Quagliarello (62) found that the white muscle of fowls and the rabbit has a slightly higher phosphorus-content than the red, but that the red is markedly richer in phosphatide phosphorus. This at once recalls to our minds Knoll, and his granules of a lecithin-like substance in the sarcoplasm of red muscle.

In the following year Costantino (18) estimated the total phosphorus, the soluble organic and inorganic phosphorus, the phosphatide phosphorus, and the protein phosphorus of smooth muscle, and of red and white striated muscle in the hen. The inorganic and soluble organic phosphorus he found to be greatest in the white muscle and least in the smooth muscle, the red being intermediate. The phosphatide phosphorus, on the other hand, was greatest in the smooth muscle and least in the white, the red again being intermediate. On autolysis, the inorganic phosphorus increased at the expense of the total soluble phosphorus, the phosphatide fraction remaining unchanged.

In order to appreciate the importance for red and white muscle of the work of the Embden school, it is necessary to consider very briefly its significance for muscle chemistry in general. About 1914, Embden (24) and his collaborators drew attention to the fact that, as had long been known, very strenuous muscular work was followed by excretion of inorganic phosphate in greater amounts than usual. They found further, that on keeping chopped muscle at 45°C. for two hours, the increase in lactic acid was accompanied by an increase in inorganic phosphate; while under certain conditions the amounts formed were equimolecular. This led to the belief that the lactic acid precursor (or lactacidogen) is a compound whose breakdown gives rise to both

these acids. Embden (20) from analogy with yeast metabolism, thought of hexosediphosphoric acid, and was finally able to show that this substance, but no other organic phosphorus compound which he tried was broken down to lactic and phosphoric acid by muscle extract. Later, Embden and Laquer (22) were able to prepare from muscle extract containing lactacidogen, an osazone identical with that prepared from yeast, and very recently Embden and Zimmermann (25a) have obtained the brucine salt of hexosediphosphoric acid. Embden and his colleagues (25) have carried out an elaborate study of lactacidogen metabolism in muscle, by determining the various phosphorus fractions under various conditions. As a method of estimating lactacidogen, they estimate the difference between the inorganic phosphate present initially, and that present after incubating the chopped muscle at 45°C. for two hours; this difference they call the lactacidogen phosphate. Under such conditions they had found that the lactic and phosphoric acids production were more nearly equivalent than under any other; we shall refer to this point later.

On examining separately the red and white muscle of the rabbit, Embden and Adler (21) found the following average figures:

	WHITE MUSCLE (BICEPS FEMORIS)	RED MUSCLE (SEMITENDINOSUS)	INTERMEDIATE (ABDUCTOR LONGUS)
	gram %	*gram %*	*gram %*
Residual phosphorus	0.2	0.3	0.25
Lactacidogen phosphorus	0.35	0.1	0.18
Total phosphorus	0.75	0.65	0.72

The residual phosphorus is the total organic phosphorus minus the lactacidogen phosphorus. It is thus seen that the quickly-moving white muscle has a larger supply of lactacidogen, while the slower but less easily fatigued muscle has more residual phosphoric acid. These same relations were found by Lyding (44) for the red and white muscle of the hen. Here the average figure for the lactacidogen phosphoric acid of the white muscle was 0.29 per cent; for that of the red 0.24 per cent; while the residual phosphoric acid for the white is about 0.10 per cent, and for the red 0.19 per cent. In a comparison of the toad with the frog, Panajotakos (56) found that the residual phosphoric acid content of the former was greater although he could find no constant differences in the lactacidogen phosphate. With the pigeon the results are interesting and fit in well with the previously-mentioned results of the present writer (50b); for the leg muscle 0.18 per cent was found for

lactacidogen phosphate, and 0.24 per cent for residual phosphate, while for the breast, the corresponding figures were 0.24 per cent and 0.46 per cent. Embden is inclined to look upon the lactacidogen content of a muscle as an index of its capability for rapid contraction, and the residual phosphorus content as a measure of its resistance to fatigue. It seems that these two qualities may be combined in certain muscles, and it is of course well known that the flight of the pigeon, while involving rapid movements, can be long sustained. In these chemical facts we begin to see a solution of the anomaly which worried the earlier histologists and physiologists—that some pigmented muscles, having the cell-structure characteristic of slow-moving muscle, yet contracted rapidly. It would be very interesting to know the lactacidogen and residual phosphorus content of one of the most discussed of these—the insect wing muscles. Further support is given to this theory by the fact established by Kahn (37) that the heart contains practically no lactacidogen but large supplies of residual phosphorus. Similarly Cohn and Meyer (17) found that the smooth muscle of the uterus contained little or no lactacidogen and, as we have seen, such muscle contains more phosphatide phosphorus than any other kind.

We must now consider the effects of fatigue on the lactacidogen content. Laquer (42) could find no decrease in lactacidogen phosphorus in excised frogs' legs which had been stimulated to fatigue; and only in certain cases of very exhausting treatment could Embden, Schmitz and Meincke (25), find a decrease upon killing fatigued rabbits and dogs. Later, Embden and Lawaczeck, however, (23) succeeded in showing under certain circumstances that the lactic acid produced in fatigue could be accompanied by a parallel increase in inorganic phosphate, which normally was immediately reconstituted into lactacidogen. As we have seen the reutilization does not seem to occur in chopped muscle.

When the red and white muscles of the rabbit were investigated separately by Cohn (16) it was found that no decrease in lactacidogen content could be demonstrated for the red. It seems, however, that a great deal of stress cannot be laid upon this observation; for in the first place the work was performed on the intact animal, and no record obtained of the amount contributed by the individual muscles. Again, all the figures obtained in these work experiments are very variable, and as it is impossible to compare under the conditions resting and fatigued muscles of the same animal, it is very possible that small changes might pass undetected. If the result is correct, it probably

means as the author suggests that the phosphoric acid in the red muscle, being liberated more slowly can be more completely reconstituted. There is no obvious change in the amount of residual phosphoric acid, and no reason to suppose that the amount of lactacidogen has been reconstituted from that source. It is a curious fact that the glycogen content of red muscle seems never to have been studied, either in the resting or fatigued condition.

This brings us to the question of the meaning of the residual phosphoric acid. Embden and Lawaczeck in 1923 (23) suggested that the high content of residual phosphorus is an index of great development of semipermeable membranes in the tissue surrounding the fibrils. They pointed out that phosphatides which would be included in the residual phosphorus, are a characteristic constituent of such membranes. They argued that if they were right in this opinion, then a high residual phosphorus should be accompanied by a high content of another substance usually associated with semipermeable membranes, i.e., cholesterol. As a matter of fact, they did succeed in finding more cholesterol in the red muscle of the rabbit than in the white, as the following figures show:

WHITE MUSCLE	RED	HEART
gram %	*gram %*	*gram %*
0.06	0.08	0.16
0.04	0.07	0.11
0.03	0.06	0.13

Later Embden and Lange (22a) suggested that the semipermeable membranes are situated in the sarcoplasm.

Adler (2) found that by keeping winter frogs at a temperature of 28°C. the lactacidogen content of their muscles was increased, and the residual phosphoric acid diminished. He suggests that one of the functions of the other organic phosphorus compounds is to act as a reserve for lactacidogen formation. More work is greatly needed on this point. It seems likely that only the phosphoric acid part of the "nichtlactacidogen" compounds can be used in making fresh lactacidogen; nothing definite is known as to the conditions under which these compounds break down, or as to what beside phosphoric acid, are their products. We know, from the observations of the histologists, that in inanition or disease, the lecithin-like substances which form the granules in the sarcoplasm may be found almost entirely converted

into droplets of fat which at later stages disappear. Again, Schaffer (69) mentions that the interstitial granules of winter frogs blacken with osmic acid: can it be possible that he happened upon the time when the phospatides were breaking down to provide phosphoric acid for the increased lactacidogen content of the summer frogs?

Another interesting case which is best considered at this point is that of Hotta's comparison (35) between the muscles of the calf and the ox. As the muscles of the former animal are paler, and its movements more active and lively, he set out in the expectation of finding more lactacidogen, and less residual phosphorus and cholesterol. Exactly the reverse proved to be the case. He correlated this with the fact that the calf was still growing, and its muscles would be expected to be less differentiated and therefore richer in sarcoplasm than those of the adult.

Embden and his collaborators have developed a technique involving the measurement of the amounts of phosphoric acid that diffuse out from a muscle suspended in Ringer's solution, for following the changes in permeability of the semipermeable membranes under different conditions. In this manner, Behrendt (5) compared the slow-moving gastrocnemius of the frog with the rapidly-moving semimembranosus, and was able to show that the latter could manifest quicker changes in permeability. He put this down to greater thickness of semipermeable membrane in the slower muscle.

We may mention here some pharmacological work of Riesser (58b) on the two kinds of muscle. He studied many drugs, but the most interesting effects were found with veratrin, physostigmin and ammonia. It was found that the contracture action of veratrin is exerted only on white muscle, red muscle being almost unaffected even by relatively high concentrations; on the other hand, red muscle, with physostigmin or ammonia shows typical well-marked contracture, while the white muscle is much less sensitive to these drugs. The author observed that the effect of veratrin on white muscle is to cause its contraction to become much more similar to that of the red muscle, and suggests that the drug by some physico-chemical action possibly on the semipermeable membranes brings the white muscle into the condition characteristic of the red muscle. This work has been followed up by some interesting studies by Riesser and Neuschloss (58c) on the effect of drugs on the permeability (measured by diffusion of phosphate) of muscle membranes, but no further comparison of the red and white muscle seems to have been carried out.

Now we have many reasons to suppose that the red striated muscle partakes partly of the characters of the white striated muscle, and partly of those of the unstriated muscle. In white muscle, the energy for movement is certainly obtained from the breakdown of glycogen and the combustion of its products. But in smooth muscle, it is said that no glycogen is present; in tonic contraction, there is little increase in gaseous exchange or heat-production, though the effort is made for a long time against a great weight and, as we have seen, the creatin theory seems inadequate. It seems clear that some mechanism of the catch and ratchet nature must be postulated; something that can hook the muscle in position and leave it there so that it uses a minimum of energy. We have seen that the carbohydrate metabolism, as indicated by the lactic acid production, of red muscle is considerably less than that of white; the gaseous exchange we shall consider later. As regards heat production, for red muscle this has been investigated only for the heart and not for skeletal muscle. Herlitzka (33a) estimated the heat liberated by the rabbit's beating excised heart, and Snyder (69a), more accurately, did the same thing with the turtle's heart, which, being cold-blooded, is much easier to deal with. These results do not help us in a comparison of red and white skeletal muscle, but it seems likely from other considerations that the heat production of the red type would be found, under certain conditions, at any rate, to be much less than that of the white; and we may suppose that in the red muscle, we have the two mechanisms combined—work performed through energy of chemical reaction, and contracted position maintained without expenditure of energy by catch and ratchet mechanism. Many suggestions have been made as to the nature of this mechanism; for example that it depends on alternate sol and gel formation (6) or on changing state of imbibition of the colloids. Whatever the physico-chemical change which takes place on the initiation of tone, there is considerable evidence that the stimulus producing the change is connected with the inorganic kations of the muscle. The recent work of Neuschloss (53) has an important bearing on this point. In 1923, this worker showed that bathing or perfusing a limb with K-free Ringer solution led to diminished tonus, while the muscles showed increased tonus in presence of increased K ions. Later, Neuschloss and Trelles (54) estimated the K content of muscle under various conditions. They found that if chopped muscle is suspended in NaCl solution, it loses K, at first quickly then slowly for about 6 hours, after which time the process comes to a standstill; the amount coming out is variable, but the amount remain-

ing "bound" within the muscle seems constant for different types of muscle. Thus they found 0.36 to 0.41 per cent of the dry substance in white rabbit muscle; 0.54 to 0.63 per cent for red rabbit muscle. This "bound" K is found in greater amount after tetanus rigor, and after contracture brought on by acetyl choline, or K, (53a), (53b). Further experiments showed that by perfusion in vivo practically all the K could be washed out, so that in the living muscle the K fraction which on death or nerve action becomes "bound," is freely diffusible. Neuschloss puts forward the theory that increased tonus depends on an increase in that fraction of the muscle K, which on death appears as "bound" K; this increase, he believes, may be brought about by stimulation of the "receptive substance" either by stimulation of sympathetic nerves, or by the action of drugs. Neuschloss found that K contracture, accompanied by increase in bound K, is not accompanied by lactacidogen breakdown, (53b) so that the contraction must be looked upon as differing from that under control of motor nerves. There is a little confusion here, however, as Lange (42a) (in Embden's laboratory) showed that under some circumstances, the addition of KCl (in concentrations similar to those of Neuschloss) led to increased lactacidogen breakdown. The explanation may be that Neuschloss worked with intact muscle, Lange with muscle brei; on the other hand, the results of Neuschloss (53c) (who found no increased breakdown) with NaCNS do not agree with those of the Embden school (Lange and Mayer) (42b) although all workers were using intact muscle. More work is needed to clear up this point. We may recall a remark of Costantino in 1915 (18) that in striated muscle the acidic ions are more than equivalent to the basic ones (Na and K) while the reverse is the case with smooth muscle, which must therefore contain sodium and potassium organically bound. He makes no mention here of red muscle, but it seems likely that it shares this property with smooth muscle.

From the considerations we have already put forward as regards the metabolism of red and white muscle, we should be inclined to expect, in spite of the presence of a possible respiratory pigment, that the oxygen uptake of the latter would be far the greater. Indeed, in 1891, Bonhöffer (9) tried some experiments on the reduction of methylene blue by the two kinds of muscle, and although his results were very contradictory, he was unshaken in his conviction that the white muscle must reduce faster than the red, and explained away his opposing results. However, in 1912 Batelli and Stern (4) stated that the oxygen uptake of red muscle is larger than that of the white; and this was afterwards confirmed by Ahlgren (3), Nilsson (55). Nilsson had shown

that the oxygen uptake of the frog's heart, as measured in Thunberg's microrespirometer, was three times as great as that of its skeletal muscle. Ahlgren in 1921, using the same technique, found the following figures for chopped rabbit muscle at 22°.[4]

TIME	RED		WHITE	
	O_2 per gram	CO_2 per gram	O_2 per gram	CO_2 per gram
p.m.	*cc.*	*cc.*	*cc.*	*cc.*
1.40–2.10	72	73	69	71
2.10–2.40	75	78	56	59
2.40–3.10	65	68	46	47
3.10–3.40	63	62	37	34

Using the methylene blue technique, he found the reduction times much less in the case of the red muscle.

Red	12.5	9	7	4 minutes
White	16.5	10	11	5

Similarly the heart-muscle of the guinea pig has a much smaller reduction time than the white skeletal muscles.

Batelli and Stern, besides studying the total oxygen uptake of the whole muscle, studied the activity of different oxidizing systems in the washed tissue. As is well known, succinic acid or phenylenediamine added to washed muscle residue, is readily oxidised; and these workers also found that the oxidation is more vigorous with the red muscle than with the white.

[4] The figures given by Ahlgren are astonishingly high. The following figures have been given to me by Mr. H. F. Holden (private communication):

O_2 consumption of rabbit muscle at 20 to 22°, during first hour, in cubic millimeters per gram

WHITE	RED
457	579
367	579
532	714
398	669
327	729
401	775

During the first hour the O_2 consumption fell off only 10 to 15 per cent for both kinds of muscle alike; this is important, as it shows that, even at the beginning of the experiment, the oxygen consumption in the white muscle was, on the average, only about two-thirds of that in the red (see below).

At least two possible interpretations of these facts are open. In the first place, it is well known that post-mortem changes take place more rapidly in white than in red muscle. None of the authors mentioned above seem to have taken any precautions such as cooling, to slow down the survival changes. In the methylene blue and respirometer experiments, where only small amounts of muscle are required, it is probable that the first readings were taken very soon after death, but where washed muscle is used, a considerable interval must elapse. It is clear that in the oxygen uptake experiments of Ahlgren the capacity of the two muscles is equal at the beginning, but that of the white shows a more rapid falling off; similarly, in the methylene blue experiments, very little difference is to be seen in the short reduction times—it is where more methylene blue is used and longer time required, that the difference becomes really noticeable.

The other possibility is that the red muscle does actually contain some oxidizable substances other than lactic acid, which may or may not be breakdown-products of carbohydrate. Such a substance might be succinic acid. It was found by the present writer (Moyle, 50a) that the chopped muscle of the ox and dog can form succinic acid when incubated in buffer solution under anaerobic conditions, the amount present rising from about 5 mgm. per cent to 25 mgm. per cent; the same thing occurred with the red muscle of the rabbit and hen, whereas in the white muscle of these animals the increase was very small. With the pigeon, the reddish leg muscle produced amounts comparable to those in the ox and dog, while in the deep purplish red breast muscle amounts nearly twice as great have been found. Precautions, such as very careful cooling before dissection and mincing, were taken to reduce as far as possible the danger of negative results in pale muscle being due to post-mortem charges. This explanation is also made unlikely by the fact that no succinic acid at all could be found in frog muscle, where the danger from post-mortem charges can be reduced to a minimum. Thus it may be that the whole question of succinic acid metabolism is more important in the red muscle. It is true that this increase, though relatively very large, is absolutely very small; but it is possible that an equilibrium is reached, and that in the living oxygenated muscle this equilibrium is continually being shifted by oxidation of the succinic acid and continual renewal. It must be remembered that no correlation of glycogen loss and lactic acid formation has ever been worked out for red muscle, nor, indeed, has the fate of the fat on fatigue or rigor been considered, as by Winfield (71) for the frog.

We have thus come to the following standpoint. In the white muscle, the fibrillary structure may be regarded as a machine adapted to produce rapid motion. The lever setting this machine in action seems to be a change in the hydrogen ion concentration, and its energy is supplied by the breakdown of glycogen through hexosephosphate to lactic acid, followed by the combustion of part of the latter. In the red muscle, the same machine seems to be present, though not elaborated or used to the same extent. We may assume that its energy is provided in the same manner. But besides this, another mechanism must be postulated, a mechanism whose state can be changed by expenditure of very little energy, and which, when in this contracted state, can maintain the muscle contracted with little or no consumption of energy. This change of state is possibly initiated and reversed by alterations in the state of ionization of potassium in the muscle. It seems probable that the greater quantity of phosphatide and cholesterol found in the red muscle, and the smaller proportion of myosin in its protoplasm, must be regarded as connected with the actual make-up, as distinguished from key or fuel, of the second mechanism.

But the problem of how the mechanism for maintenance of position and the mechanism for change of position became incorporated in one and the same muscle "might admit" as Sir Thomas Browne would have said "of a wide solution; and shall tax hardly the Judgment of Posterity."

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THE DETERMINATION OF SEX IN ANIMALS[1]

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The first and most fundamental fact bearing on sex determination brought to light, was a difference in cellular constitution of male and female animals. This fact was known for years before its significance for sex was realized. Henking (1891) described a chromosome-like element in the male germ cells of an insect, which exhibited peculiar behavior during the reduction divisions preliminary to sperm formation. He observed that but half the sperm cells received this body, while the other half were without it. Thus, two classses of spermatozoa are formed. Similar observations on other insects were reported by Paulmier (1899), Sinéty (1901) and McClung (1902). The last named was the first to suggest that the peculiar chromosome was probably concerned with sex determination. Wilson (1905–06) and Stevens (1905) demonstrated that the females of various insects have one more chromosome than the male, and that consequently the diploid chromosome group of this sex in some animals has two X-chromosomes (the term first applied by Wilson, 1909, to the odd chromosome of the male), the male but one. In the course of gamete formation, the eggs are all alike but the sperm are of two classes with respect to the X-chromosome. Thus cytological observation demonstrated a fundamental morphological difference between the sexes in some animals which distinguishes every cell in the organism.

A second important advance, was the correlation of chromosome behavior during gametogenesis with the behavior of the Mendelian factors in heredity (Sutton, 1903; Wilson, 1902), and the assumption (now an established fact) that the chromosomes are the bearers of the hereditary units. For, as we shall see later, the study of sex inheritance has thrown a flood of light upon the problem of sex determination, and the relation of the latter to certain chromosomes. We shall discuss first some of the evidence that sex is dependent upon chromosome constitution.

[1] The writer takes this opportunity of expressing his obligation to the Biological Laboratory, Cold Spring Harbor, for laboratory facilities during the summer of 1925, when much of this paper was written.

MALE DIGAMETY. This term denotes the condition in which the male forms two kinds of sperm cells and female but one kind of egg. It is by far the commonest type met with among animals and, in fact, is characteristic of most animal groups except the Lepidoptera (moths and butterflies) and birds.

One-half of the sperm cells, in forms exhibiting one type of male digamety, contain an X-chromosome and hence are female-producing under ordinary conditions, while half lack the X and are male-producing. The cells of the female contain one more chromosome than the male and consequently the total chromosome number is even in the female and odd in the male. All of the mature eggs are alike, each receiving a haploid chromosome series including an X, during maturation. The unpaired X-chromosome of the male has no mate, and during gametogenesis enters but half the sperm. The latter thus forms two classes numerically equal, one-half containing an X and the other half lacking this chromosome. The sex of the zygotes is thus seen to depend upon the class of sperm that fertilizes the egg. There should result in each succeeding generation equal numbers of XX (female) and XO (male) individuals. The bisexual condition is thus perpetuated by a chromosome mechanism. It should be reiterated that the condition described is well established by cytological observation and experimental breeding. The two types of sperm, X and no-X, with the chromosome number clearly visible, can be observed in the living spermatozoa of the nematode worm, Ancyracanthus. It has been possible to establish the entrance into the egg of both classes of sperm, and to determine that the sex of the resulting individual depended upon the type of sperm fertilizing the egg. The X-bearing sperm resulted in females, the no-X giving rise to males. This was first demonstrated through the researches of Mülsow (1912).

Besides the type of male digamety in which the female has one more X-chromosome than the male, there is another type in which the chromosome number in the two sexes is the same, but the mate of the X-chromosome in the male differs from it in several features, and consequently is known as a Y-chromosome (Wilson, 1909). The sex formulas thus become XY = male and XX = female. A large number of animals, including man, exhibit this type of digamety. During spermatogenesis in this type, the X and Y of the male join just as do the two X chromosomes of the female, and are then separated at the reduction divisions so that half the sperm receive X and half Y. All of the mature eggs receive a single X. Fertilization of an egg by the X-bearing sperm

will produce a female, XX, and if a Y-bearing sperm enters the egg, the male combination XY results. In principle, the XX-XY type is the same as the XX-XO type. In fact, breeding experiments have clearly demonstrated that the Y chromosome lacks sex factors, i.e., it exerts no influence on sex (Bridges, 1914, 1916). Insofar as having any influence on sex is concerned, the Y might just as well be absent, as in the XO type. In fact Wilson (1910) observed that the Y is absent in some specimens of Metapodius.

There are numerous interesting modifications of the XY type (Wilson, 1905; Stevens, 1910; Payne, 1912; Painter, 1922; Schrader, 1922) which occur in various animal groups, but the end result for sex is the same, hence such modifications need not detain us here. In both types of male digamety, the single X-chromosome of the male is derived from the mother. This chromosome, therefore, alternates between the sexes in successive generations.

Morgan (1911) and others demonstrated by breeding tests that the X always passes from the male to the female in the production of females, and from the female to the male in the production of males. We shall see later that this fact has an important bearing upon the mechanism involved in criss-cross inheritance of sex-linked characters.

Female digamety. Two animal groups, Lepidoptera and birds, present an interesting situation with regard to their chromosomal constitution. For genetic and cytological evidence proves beyond doubt that in the moths and butterflies, the sex chromosome situation is exactly the opposite of that characteristic of the XX-XO (or Y) type. In these insects the eggs are of two kinds, whereas the sperm are all alike, and the phenomenon is known as female digamety. Birds also exhibit this type of digamety as shown by breeding tests (Pearl and Surface, 1910, and numerous others) but to date cytological confirmation is lacking, due to certain technical difficulties inherent in the material. But so far as direct observations go of the chromosome complement of birds, it agrees with the breeding results (Hance, 1924; Shiwago, 1924).

The sex-chromosomes of Lepidoptera were first demonstrated by Doncaster (1910–1915) and Seiler (1914–1922). In the male moth all of the chromosomes are paired, and thus evenly distributed to the sperm so that all of the latter are alike. In females, however, two types of eggs are formed corresponding to the XO-XX type. For example, Seiler found that in Fumea casta the male diploid chromosome number is 62, the female number 61. All the sperm receive 31 chromosomes but the

eggs are of two types, one containing 31 chromosomes, the other 30 chromosomes. It is obvious that at fertilization individuals with 62 chromosomes (males) and individuals with 61 chromosomes (females) will occur in equal numbers. The females of certain Lepidoptera are said to be XY instead of XO in constitution.

An interesting situation arises here, in that the sex-chromosome relations in cases of female digamety are exactly the reverse of those in male digamety. It becomes something of a problem to correctly designate the sex-chromosomes. In the Lepidoptera, in as far as the chromosome situation is concerned, XX = ♂ and XO = ♀ but in male digamety it was stated that XX = ♀ and XO (or XY) = ♂, so the symbol X does not mean the same thing in the two types. Because of this fact, cytologists and geneticists have employed a different terminology for the sex-chromosomes in cases of female digamety, and have substituted the letters W and Z for X and Y.

Both types of digamety employ the same general type of chromosome-mechanism to insure the production of sex digamety in each generation. Moreover, the phenomenon of sex-linked inheritance is similar in principle in both types.

We have indicated certain fundamental cytological differences in cellular constitution of the sexes, and expressed the current zoölogical belief that certain chromosomes function in some manner as sex determiners. It will be of interest at this point to present some of the evidence from experimental breeding, that the so-called sex chromosomes are really vitally concerned in sex determination.

SEX-LINKED INHERITANCE. The evidence from genetics has shown beyond doubt that the sex chromosome carries many other factors than those associated with sex. The behavior in inheritance of characters, the factors for which are carried by the sex chromosome, is peculiar, because they are linked, so to speak, with sex. By this is meant that, in inheritance, those factors which are carried by the sex chromosome are distributed by the same mechanism that determines the normal distribution of the sexes. Study of sex-linked inheritance clearly indicates the importance of the sex chromosome as a sex differentiator (Doncaster, 1908–1914; Morgan, 1910; Pearl and Surface, 1910).

Numerous well-authenticated cases of sex-linked inheritance have been worked out in animals with male and female digamety (Morgan, 1910; Bateson and Punnett, 1911; Goodale, 1911; Sturtevant, 1912; Davenport, 1912). The classical case reported by Doncaster (1908–1914)in the currant moth (Abraxas) will serve as an illustration. It will

be recalled that moths exhibit female digamety, i.e., are WZ—ZZ in chromosome constitution.

Doncaster found that in this moth, the wild species (grossulariata), has a variety, known as lacticolor, which differs from the former by having less black pigment on the wings. When a dark grossulariata male is mated to a light lacticolor female, both sons and daughters are dark, i.e., of the grossulariata type. If these are inbred all the sons are dark, whereas half the daughters are dark and half light. The distribution of the Z-chromosome furnishes the mechanism whereby it is possible to explain sex-linked inheritance in this moth.

In the reciprocal cross, lacticolor male by grossulariata female, the daughters are lacticolor like the father, and the sons are grossulariata like their mother (criss-cross inheritance). When the hybrids of such a cross are inbred, the result is lacticolor males and females, and grossulariata males and females in equal numbers.

This type of sex-linked inheritance can be explained on the assumption that the factor for lacticolor is carried by the Z-chromosome. It is evident that in this case the genetic evidence calls for a chromosome mechanism in which the female is heterozygous for sex, since the eggs carrying the grossulariata factor develop into females, the others into males. As stated before, the cytological investigations of Doncaster and Seiler have demonstrated that in Lepidoptera the female produces two kinds of eggs in regard to chromosome constitution, and the male but one kind of sperm. Thus we see that the genetic and cytological findings agree. Many other similar cases are known and the same situation holds true for male digamety, except here the distribution of the sex-linked characters accords with the fact that the male is heterozygous for sex. Occasionally, however, the predicted distribution of sex-linked characters to the offspring does not come out according to expectation, and the theory seems at first sight not to fit the facts. However, study of these exceptional cases has brought to light a unique and most striking demonstration of the relation of sex to chromosomes. The phenomenon is known as nondisjunction.

Non-disjunction. One of the most interesting and suggestive investigations of the mechanism of sex inheritance, and the relation of sex chromosomes to sex distribution, we owe to Bridges (1913–1916). His studies were concerned with the origin of certain exceptional individuals which appeared from time to time in cultures of the fruit fly, Drosophila. Ordinarily, in the case of sex-linked characters, when a female with the recessive character is mated to a male with the domi-

nant character, all the females of the F_1 generation exhibit the dominant sex-linked character, and all the males the recessive character. The reason for this is that the male receives his X-chromosomes from his mother, whereas the father transmits his X-chromosome bearing the dominant allelomorph to his daughters. Bridges studied the inheritance of the factor for vermilion eye-color. The vermilion factor V is borne by the sex-chromosomes, and since the males from crosses between vermilion females and normal red-eyed males receive their only X-chromosome from the mother, they should all be vermilion-eyed. The females from such a cross receive from the father an X-chromosome bearing the dominant allelomorph of V, consequently they should all be red-eyed. In the vast majority of cases this is actually so, but occasionally a vermilion female or a red-eyed male appears as an exception. The study of these exceptional flies led to the discovery of non-disjunction, for their occurrence could only be explained on the assumption that occasionally the two X-chromosomes failed to separate at the reduction divisions during gametogenesis, both passing out of some of the eggs of the vermilion-eyed individual into the polar-body, or conversely, both remaining in the egg. If the two vermilion-bearing X's should remain in the egg then such an egg fertilized by a Y-bearing sperm (Drosophila is of the XX-XY type) would give rise to a vermilion-eyed exceptional daughter. Likewise the no-X egg fertilized by the X-bearing sperm of a red-eyed male would give a red-eyed exceptional male. The cells of the vermilion daughters would contain two X's and a Y-chromosome, thereby differing from the ordinary daughters which contain but two X's. Thus these females possess three sex chromosome (XXY) instead of a pair (XX) and consequently at the reduction division two chromosomes will pass into one cell and one into the other making four classes of eggs possible:

X XY XX Y

If an XXY vermilion daughter is mated to a red-eyed male the progeny produced by the X-bearing sperm would have the following sex chromosome constitution: (Morgan, Sturtevant, Muller, Bridges, 1923)

(1)	(2)	(3)	(4)
XX	XXY	XXX	XY
♀ Red	♀ Red	dies	♂ Red (exception)

The same series of eggs fertilized by the Y-bearing sperm (male produc-

ing under ordinary circumstances) gives the following classes of individuals.

(5)	(6)	(7)	(8)
XY	XYY	XXY	YY
♂ ver.	♂ ver.	♀ ver. (exception)	dies

The exceptional vermilion females (7) are of the same chromosome constitution as the original fly and consequently should repeat the process and be non-disjunctional. Breeding tests show that such is actually the case. The red-eyed male (4) should not give exceptional results when mated to normal females. This is obviously so because it has the constitution of normal males.

The females are of two kinds, half (1) should behave like normal females heterozygous for vermilion, and the other half (2) should give exceptions. Repeated breeding tests confirmed the expectations.

The vermilion male (5) and (6) are not alike: one kind (5) is normal, and the other (6) has two Y-chromosomes. Some sperm of the latter would be XY and give daughters which would not themselves be exceptional, but females with a formula XXY should produce exceptions. By breeding experiments, Bridges showed that from half of the males of class 5 and 6 daughters are produced that give non-disjunction.

The genetic results confirm to a nicety the hypothesis that the exceptional individuals are due to non-disjunction of the sex chromosomes caused by the presence of a Y-chromosome in the female. Cytological investigation of non-disjunctional females shows that such females do contain a supernumerary Y-chromosome. As a result of this brilliant investigation it is conclusively demonstrated both by experimental breeding and cytological observation that an abnormal distribution of all the sex-linked characters goes hand in hand with an identical abnormal distribution of sex chromosomes.

L. V. Morgan (1922, 1925) has described another case of complete reversal of the ordinary criss-cross inheritance of recessive sex-linked characters in a line of Drosophila. We saw in discussing the work of Bridges, that in ordinary sex-linked inheritance the recessive sex-linked characters of the mother are transmitted to the sons, while the daughters show the dominant allelomorphic characters of the father. In the example studied by Mrs. Morgan, the reversal was assumed to be due to union of the two X-chromosomes of the mother so that they behave at reduction as a single body (XX-linkage). Cytological examination of such flies showed that the eggs of the females do have two united X-chromo-

somes. Thus the cytological evidence verified the genetic deduction, and affords further evidence of the relation between sex-chromosomes and the distribution of sex characters.

RELATION OF NON-DISJUNCTION AND XX-LINKAGE TO SEX DETERMINATION. The work of Bridges and Mrs. Morgan is of significance from the standpoint of sex chromosomes and sex-determination. The work of Bridges especially has clearly shown that sex is not predetermined in the gametes but is determined by the chromosome constitution established in the egg at the time of union of the gametes. It was previously stated that the X-bearing sperm in male digamety is the female-determining one, and the Y or no-X sperm the male-determining element under normal conditions. However, in certain cases of non-disjunction these conditions are reversed, and the X-bearing sperm becomes the male determining one, and the Y-bearing sperm the female determining body. This apparent contradiction instead of rendering the argument void, offers the best proof yet obtained that it is not the sperm or egg as a whole that determines sex, but the X-chromosome. The Y-class of sperm, normally male-producing, becomes female-producing if it fertilizes a non-disjunctional egg containing two XX's which have failed to disjoin in oögenesis. Conversely, the X-class of sperm, normally female-producing, becomes male-producing if it enters an egg of the Y class.

The fact stated become apparent if the diagrams of the classes of eggs formed by a non-disjunctional female are studied. These are: egg X, egg XY, egg XX, egg Y. The sperm are of two classes X and Y and the X bearing sperm is female-determining, because the presence of two XX's in an egg always results in females. Thus (Wilson, 1925):

1 Egg X fertilized by sperm X = XX ♀
2 Egg XY fertilized by sperm X = XXY ♀
3 Egg Y fertilized by sperm X = XY ♂
4 Egg XX fertilized by sperm X = ? zygote non-viable

It will be noted that in (3) the egg Y fertilized by an X sperm (normally female producing) results in a male XY in constitution. The same is true when the four types of eggs are fertilized by Y-bearing sperm.

1 Egg X fertilized by sperm Y = XY ♂
2 Egg XY fertilized by sperm Y = XYY ♂
3 Egg Y fertilized by sperm Y = ? zygote non-viable
4 Egg XX fertilized by sperm Y = XXY ♀

In (4) the Y-bearing sperm (male-determining normally), if it enters an egg containing XX, becomes female-producing. The evidence seems conclusive that the sex of the individual depends upon the chromosome combination, no matter how it is established. L. V. Morgan's study of XX-linkage demonstrated the same. As a result of the work mentioned, together with some other, which there is not space to discuss here, zoölogists believe that there is but one kind of X-chromosome and that this in itself is neither male-determining nor female-determining, but stands in such a relation to the developing organism that when it is single (XY) it swings development toward "maleness," and when double (XX) it tips the balance in the direction of "femaleness." In other words, the egg is capable of producing either a female or a male individual depending on whether it contains more or less of X substance. This point of view was clearly expressed by Wilson (1909 and 1911) fifteen years ago, as a result of his cytological studies, and long before non-disjunction and XX-linkage were known. (See also Wilson, 1925.)

In the foregoing section we have attempted to present the cytological and genetic evidence that the sex of an individual is dependent upon its chromosomal constitution. In the three following sections this concept will be applied to the elucidation of some sexual phenomena occurring in animals, which at first sight seem decidedly at variance with the facts just outlined.

PARTHENOGENESIS AND CYCLICAL SEXUALITY. Any theory of sex production or determination must take into consideration haploid and diploid parthenogenesis, so common among certain animal groups. At first thought it may seem that parthenogenetic reproduction, and especially the strange phenomenon of cyclical sexuality, is to prove the rock upon which the chromosome theory of sex is to be wrecked, but such is not the case. Indeed, it is in these exceptional types of sex behavior and production that the theory receives its strongest support.

Among animals which reproduce both by means of fertilized eggs and by parthenogenesis, two general types are known. In one type but one kind of egg is produced which is capable of being fertilized and also of developing parthenogenetically. Animals of the second type produce two kinds of eggs, only one of which is capable of developing parthenogenetically, the other requiring fertilization, or at any rate is capable of being fertilized. The Aphids, Daphnids and Rotifers are the groups in which these types occur. For illustrating the relation of parthenogenesis to sex production and determination, we shall use the aphids, since their chromosome cycle has been worked out so thoroughly by Baehr (1909), Morgan (1909–1912), Stevens (1909–1910) and others.

In some aphids there occurs a succession of parthenogenetic females, and the length of this portion of the cycle, i.e., the number of generations has been shown to depend upon environmental conditions. At the end of the series males and sexual females appear. These females have the double number of chromosomes. The males which appear have one less chromosome than the females (Baehr, 1909; Stevens, 1909–11). Morgan (1909) was able to demonstrate that in the case of the closely related Phylloxera caryaecaulis, a similar reduction in number of chromosomes occurs, and that it is due to extrusion of whole chromosomes into the polar bodies during oögenesis, so that the individuals arising from such eggs (males) had one less chromosome than females. During gametogenesis of the male, one cell gets the unpaired X-chromosome, and this cell after an ordinary division, produces two functional sperm, both alike. The cell lacking the X degenerates. The sexual egg gives off two polar bodies, and then contains the haploid number of chromosomes including one X. If such an egg is fertilized by the functional X-bearing sperm it will give rise the following year to a stem-mother which in turn will be the starting point for a new line of parthenogenetic females.

Morgan's work (1909) on the chromosome relations of the phylloxeran of the hickories is a striking demonstration of the close dependence of sex determination to chromosome constitution in the complicated life cycle of these parthenogenetic forms. The chromosome cycle is as follows: There are eight chromosomes in the species Phylloxera caryaecaulis, and of these four are apparently concerned with sex determination; they are the only ones discussed here. The fertilized egg gives rise to a female called the stem-mother. These females attach themselves to the leaves of the hickory and a gall forms around them, in which they lay their eggs. The eggs extrude a polar body, but the division is not reductional because all eggs have the four sex chromosomes. The eggs develop parthenogenetically into winged migrant females. These are of two kinds: those which lay large eggs and those which lay small eggs. All females which are derived from the same stem-mother lay the same kind of eggs. Thus there are two kinds of stem-mothers with respect to chromosome constitution: the female-producing stem-mothers are XxXx and the male-producing stem-mothers are XxXx′. The migrant female XxXx produces large eggs which throw off a polar body, but do not undergo chromosome reduction. The resulting egg develops without fertilization into a small sexual female. The other type of female lays small eggs in which the large X's and the small X's

conjugate. One of each of these chromosome pairs is then thrown out into the polar body so that two types of eggs are produced, Xx and Xx′, and these develop without fertilization into small males. In the sexual females, a true reduction division takes place, so that her single egg is of the chromosome constitution Xx. The males on the other hand produce sperm cells, half of which have none of the sex-chromosomes. Sperm of the latter type degenerate, so that only female producing sperm remain. When these fertilize the sexual egg, the resulting eggs are either XxXx or XxXx′, and give rise to the corresponding type of stem mother as indicated by the symbols. Thus the cycle is completed, and it affords an amazing confirmation of the conclusions regarding the relation of chromosome to sex, based on the condition existing in ordinary forms.

There is one point however which is not entirely clear, and that is the manner in which the male-producing and female-producing stem mothers are differentiated. Some sort of differential division must occur during the maturation process of the eggs from which such individuals arise but the evidence on this point is not so clear as could be desired. Morgan (1912) has described several peculiarities of chromosome behavior in the male-producing eggs, which may indicate that a portion of the X-chromosome is thrown out of the cell during the maturation of the stem-mother's egg. Morgan has assumed that one pair of sex-chromosomes is heterozygous in a factor indicated by priming one the X's.

The case of the bee and certain other Hymenoptera offers contradictions to the usual chromosome mechanism of sex production and determination. Bee eggs which develop parthenogenetically give rise only to males, whereas fertilized eggs develop into females. Cytological investigation shows that the queen bee possesses 32 chromosomes. Sex chromosomes are present, but cannot be distinguished from the other chromosomes. During the process of egg ripening the chromosome number is reduced to 16. If such an egg is fertilized, it develops into a female; if fertilization does not occur the egg develops into a male with the reduced number of chromosomes, i.e., 16 and possessing but one X-chromosome. Cytological study has shown that a non-X type of sperm is not formed. The question arises as to what becomes of the no-X sperm since the X-carrying type will give a female, and only females arise from fertilized eggs. Meves (1907) discovered that no reducing division occurs in the gametogenesis of male bees. The first division is abortive and affects only a portion of the cytoplasm, the

second division is more complete and results in two unequal nucleated cells, but one is very small and degenerates. All of the functional sperm are of one kind and contain sixteen chromosomes (Petrunkevitch, 1901; Meves, 1907; Nachtsheim, 1913).

We may justly conclude that parthenogenesis and cyclical sexuality in animals, despite the extreme complication of the life cycles and its manifold types (e.g., there are parthenogenetic eggs which give females without reduction, others which give females after reduction; some which give males with reduction, and others which give both sexes with and without reduction) affords some of the best evidence yet presented for the intimate relation between chromosome constitution and sex. For in all of these different conditions, cytology has shown reasons for believing that different mechanisms are present in the different groups which determine the right combination of the X-chromosomes to produce the resulting sex. It is true that numerous gaps in our knowledge exist, and the reviewer has no desire to minimize them, but the evidence at hand is such that we may feel sure that when our information of parthenogenetic forms is complete, there will be no great surprises.

In many animals showing cyclical sexuality such as Aphids, Rotifers and Daphnids, the transition from parthenogenesis to sexual reproduction can be more or less controlled by regulating certain features of the environment. For instance, Whitney (1914, 1917, 1919) has shown that sexual reproduction in Rotifers can be postponed, or even prevented, by a variety of chemical substances in the culture medium, or it may be hastened by the use of green protozoan food, or by increasing the oxygen content of the water (Shull, 1918). Banta (1918–24) has demonstrated that in Cladocera, temperature, food and excretions in the medium affect the advancement or postponement of the sexual phase.

However, it should be held constantly in mind when dealing with cyclical sexuality that these environmental changes which are thought by many to be sex-determining, are really not sex-determining at all when this term is properly employed. For illustration we may take the case of Rotifers. According to Whitney (1914), parthenogenetic females if fed on a poor diet of Polytoma, continue to produce parthenogenetic females like themselves, and parthenogenetic reproduction continues indefinitely. If, however, such females are fed quantities of the protozoan Euglena, they lay eggs which develop into individuals which if early fertilized become sexual females, but if not fertilized lay small eggs. If these eggs develop without fertilization they become males; thus the same female becomes either a sexual female, or a female

that gives rise to males. In other words, as Morgan (1919) has pointed out, the change in food (Polytoma to Euglena) induced the appearance of a kind of female that is either a producer of eggs that become males, or a sexual female should she early meet a male.

It is probable, though not yet demonstrated, that these environmental changes produce their effects by inducing changes in the chromosome mechanism of sex production. Indeed, Morgan (1919), Goldschmidt (1920) and others insist that the rhythm of cyclical sexuality is not a problem of sex determination at all, since the rhythms exhibited by the Rotifers and Daphnids do not determine one or the other sex, but either females or both sexes. In the words of Goldschmidt (1923), "The rhythm is nothing but a period which rules the maturation of the egg (as well as the possibility of parthenogenesis) which then itself automatically determines sex." The most recent discussion of this problem is that of Shull (1925), to which the reader is referred.

GYNANDROMORPHISM. In many species of insects, and especially in the Lepidoptera, an anamolous sex situation occasionally arises, where the individual exhibits male characters on one side of the body and female characters on the other. Such individuals are known as gynandromorphs, and the phenomenon has an intimate bearing on the relation of chromosomes to sex.

Gynandromorphs are really mosaics, in which the essential features of the two sexes are sharply delimited from each other in different regions of the body. The mosaic formations vary, sometimes the right and left halves of the body are of different sex, including the gonads, in other cases, the anterior portion of the animal presents the characters of one sex, the posterior part those of the other sex, and still other cases are known where male and female parts (always definite and distinct) are arranged in every conceivable manner.

In the solution of the problem presented by such sexually aberrant individuals, we find again that chromosomal constitution is the essential feature. It was early recognized by investigators of gynandromorphism that irregularities in distribution of the X-chromosomes could account for the sex mosaics. The difficulty lay in proving the assumption.

The most decisive evidence yet presented in the solution of this problem is that of Morgan and Bridges (1919) on the sex mosaics of Drosophila. Their extensive work on sex-linked and other characters of the fruit fly, enabled them to trace certain characters known to be carried by the sex-chromosome, in the gynandromorph, and thereby

get a clue as to the chromosomal make-up of such individuals. Sex-linked characters are carried by the X-chromosomes. If gynandromorphism occurs in individuals which are hybrid in respect to one or more of these characters, the male and female parts of the hybrid will indicate the chromosome constitution of such parts. Several such cases were studied. For example: a gynandromorph appeared which carried sex-linked characters, the abdomen and thorax were male, but the head was entirely female. The male parts all showed the usual sex differences, and *also the sex-linked recessive character*, yellow body color. If we recall for a moment that a recessive sex-linked character borne by one X-chromosome cannot appear in the presenc of its normal allelomorph borne on the other sex-chromosome, we have a clue as to the chromosome constitution of the male and female parts. In this case it must be the yellow-bearing X-chromosome that gives the yellow, male, side of the gynandromorph, and since the female characters arise when two X's are present, both must be present in the female parts, which will thus be of normal (wild type) color, because the gene for wild type color is dominant to yellow. Such a gynandromorph must have arisen, therefore, at a very early nuclear division of the cleaving egg, in which one daughter X-chromosome failed to pass into one of the daughter nuclei. Morgan and Bridges were able to demonstrate that both nuclei, the one containing XX, and the one with XO (or Y) constitution, contain chromosomes derived from both parents. This was done by making one of the original parents homozygous for a definite character carried by an autosome. If all of the chromosome of the fertilized egg divided normally except the X-chromosome, the autosomal character should be present in both nuclei and therefore present in male and female parts of the mosaic. This was found to be the case. On the basis of this convincing genetic evidence, Morgan and Bridges advanced the view that the great majority of gynandromorphs result from the accidental elimination of one of the sex chromosomes at some early division of the fertilized egg.

Three other explanations of gynandromorphism have been offered, all based upon the chromosome constitution of the individual. The three theories are: 1, Boveri's partial fertilization theory (Boveri, 1888, 1915); 2, Doncaster's (1914) double nuclear fertilization theory; and 3, Morgan's (1905) supernumerary spermatozoan idea. Suffice it to say that all of these theories are based on the idea that irregularity of chromosome distribution is the cause of gynandromorphism, and all have some cytological basis in fact.

The genetic evidence is conclusive that gynandromorphism in insects is due to a disturbance of the X-chromosome mechanism. However, there are a few authentic cases of gynandromorphic birds which are not so easily explained. Weber (1890) and Poll (1909) have both described finches in which the right side of the body was male, and left female, in respect to plumage character and sex glands. Bond (1913–1914) has described a pheasant which had female plumage on the right side of the body and male plumage on the left. The reproductive glands were a mixture of male and female parts.

The explanations advanced to account for gynandromorphism in insects do not cover these cases satisfactorily, and for this reason, the type of plumage characteristic of female birds is dependent upon hormones emanating from the ovary and circulating in the blood-stream. If a hen is ovariotomized (Goodale, 1916) she develops typical cock plumage after the first molt. On the other hand, if a cock is castrated, he does not change his plumage, but if an ovary be transplanted after castration (Goodale, 1916; Pezard, 1918; Zawadowsky, 1922–1923) he loses the rooster plumage and develops that of the hen. Bilateral gynandromorphism in birds, with respect to plumage, is difficult to explain, because it is obvious that the sex hormones are circulating in all parts of the body. Pezard (1922) has offered a tentative explanation of gynandromorphic birds. He assumes that in such a condition there is a somatic and hormonic abnormality. In normal animals both sides of the bird's body react to the threshold quantity of female hormones, and female plumage is symmetrically developed. If we assume that the threshold quantity is abnormally high for one side (possibly due to differences in chromosome constitution of the cells of that side) and that at the same time only the threshold quantity of female hormone is produced in such a bird, a bilateral gynandromorph, with respect to plumage, will result. The quantity of hormones produced will be able to feminize only that side of the animal which is normal. The abnormal side (with high threshold requirements) will reveal the male plumage.

HERMAPHRODITISM AND THE CHROMOSOMAL HYPOTHESIS OF SEX DETERMINATION. Many animal groups exhibit the phenomenon of hermaphroditism, in which both eggs and sperm are produced by the same individual. There are numerous types and gradations of hermaphroditism, and some of the most curious and interesting sex conditions known occur in monoecious forms.

Certain animals, like the earthworm, possess both male and female sex

glands, but these are located in different regions of the individual's body. Both eggs and sperm are ripened but self-fertilization does not occur. In other groups of worms, self-fertilization does occur. Some Nematodes and Molluscs exhibit a type of hermaphrodition in which a male or female animal develops eggs in the testis and sperm in the ovary at certain times, with the result that one or the other sex becomes superfluous and practically disappears. Other Nematodes and Molluscs are first males and function as such, then later become functional females, and several variations of this type are known but they need not detain us here, since for our problem one kind of hermaphrodition will serve as well as another. The problem with which we are faced is to explain how an individual can produce both eggs and sperm, simultaneously or consecutively.

At the outset of our discussion a difficulty arises, for despite the fact that the study of hermaphroditism offers great possibilities for the elucidation of sex determination and differentiation, the field is practically untouched. Our knowledge of hermaphrodition is the least satisfactory of any in the entire field of sex. It is obvious that it offers difficulties for the chromosome hypothesis, especially the morphological form in which the latter is usually considered. As we shall see later, it is necessary to be clear as to just what the chromosomal hypothesis implies when studying hermaphroditism.

But four cases of sex-chromosomes occurring in hermaphroditic animals are known, and two of these are regarded by some writers as doubtful, and the third is an amazing contradiction to the theory. Thus, we have but one clean-cut case of a direct relation between chromosome behavior and sex, in monoecious animals.

The Nematode, Angiostomun nigrovenosum, has two phases in its life-cycle: a free living generation in which the sexes are separate, and a parasitic generation which lives in the frog's lung and is hermaphroditic. Schleip (1911) and Boveri (1911) independently investigated the cytological conditions existing in this worm. The females of the free living forms (non-hermaphroditic), possess twelve chromosomes, and they produce eggs with six chromosomes after reduction. The males have only eleven chromosomes and produce two kinds of sperm, half with six and half with five chromosomes. However, only the class of sperm with six chromosomes is capable of fertilizing the eggs because the cells of the animals of the succeeding generation always have twelve chromosomes. This generation is hermaphroditic and produces both eggs and sperm, the same sex gland giving rise to both. All the eggs at

maturation exhibit the reduced number of chromosomes, i.e., six. In the sperm of the male, two X-chromosomes are unpaired, but the remaining autosomes pair to form five bivalents. These are separated at the first division, consequently the sperm each receive five autosomes and one X-chromosome. However, in one cell the X is eventually cast out and lost. The hermaphrodite thus forms two kinds of sperm, half with five and half with six chromosomes. As a result of this mechanism of chromosome distribution it becomes apparent how the male of the free living generation has eleven and the female twelve chromosomes. The chromosome mechanism is fairly clear in this case and the facts fit in with the chromosome theory. However, it should be pointed out that this case is really not typical of hermaphrodition in general, because: 1, the hermaphrodite generation is really a modified female; 2, the species is hermaphroditic for only a portion of its cycle; and 3, it is not clear why the parasitic generation with twelve chromosomes (XX) should not be a true female instead of an hermaphrodite.

Demoll (1912) and Zarnik (1911) have described sex chromosomes in the snail, Helix pomata, and the pteropod mollusc, Creseis, both of which are hermaphrodites. Zarnik's description of the chromosome-cycle in Creseis is remarkable. The animal possesses twenty chromosomes. In the spermatogonia there are sixteen autosomes, two large X-chromosomes and two small X's. The first division is reductional, resulting in two secondary spermatocytes each with eight autosomes, and one large and one small X-chromosome. At the second or equational division, the small X does not divide but passes intact into one sperm mother cell. Thus two kinds of sperm are formed, one with eight autosomes and one X, the other with eight autosomes and two X-chromosomes. The sperm possessing but one X degenerate. In the oögonia there are sixteen autosomes and four small sex-chromosomes. Following reduction all eggs are alike and contain eight autosomes and two small sex-chromosomes. Fertilization always results in an hermaphroditic individual with sixteen autosomes, two large and two small X-chromosomes.

The case seems clear enough at first sight but unfortunately when closely scrutinized presents several difficulties. The same is true of Demoll's description of the sex-chromosomes of Helix. Neither of these investigations presents us with clean-cut evidence similar to that of Schleip and Boveri.

The fourth case of sex-chromosomes described in an hermaphrodite (?) is Junker's (1923) description of the chromosomes of Perla marginata,

a pseudoneuropteran. The sexes are separate in this form and the females present nothing unusual. The males, however, possess an ovarial-like structure situated between the two testes, anteriorly, which consists of egg tubes containing rudimentary and non-functional egg-like cells. The diploid chromosome number in spermatogenesis is twenty-two of which twenty are arranged in ten pairs, while the two large dissimilar elements remain unpaired. They are the two X-chromosomes, X and X′. Two kinds of sperm are formed, half possessing twelve chromosomes and half with ten chromosomes. The diploid number in the female is twenty-four. All the mature eggs are alike and contain twelve chromosomes. Now the peculiar thing about this case is that the cells of the "male ovary," i.e., the ovary-like body in the male, have twenty-two chromosomes, twenty of which are paired, and two unpaired X's. That is to say, the male number of chromosomes is present in the egg-like cells. These cells behave like egg cells except that development is soon arrested and they degenerate. Here we have the astonishing condition of oöcyte-like cells, and a structure closely resembling an ovary, produced in the presence of the chromosome number characteristic of the male—a condition just the reverse of that described for Angiostomum.

There are several significant facts to bear in mind before accepting Junker's radical conclusion that chromosomes have nothing to do with sex determination. Some of the points are:

1. It is significant that the ovarial-like structure is confined to the male.

2. The so called oöcytes or "male-eggs" never become functional but invariably degenerate in early developmental stages.

3. The evidence is far from clear that the structure under discussion is really ovarial, and that the oöcyte-like cells are really immature ova. There are several cases on record where the male sex cells during gametogenesis take on the appearance of egg cells although eventually giving rise to spermatozoa. This is notably true of certain Chilopoda, e.g. Scolopendra and Lithobius (Blackman, 1905; Swingle, 1922).

4. Even if the structure in Perla is a rudimentary ovary, with oöcytes containing the male complement of chromosomes, it is not necessary, in order to account for the phenomenon, to throw overboard the vast amount of convincing evidence that has accumulated showing the determinative effect of chromosome constitution upon sex. It is obvious that there is something lacking in the make-up of the oöcyte-like cells, because they always degenerate. The probable explanation of this

case is that there is some disturbance of the normal balance between the sex factors in the males whereby the ovarial-like structure arises. We shall see later that the sex of cases like Perla is just as dependent upon chromosome and genetic constitution as any other.

The whole subject of the chromosomes of hermaphrodites evidently calls for further investigation. Wilson (1925) has pointed out it should be kept in mind in such an inquiry, that the true hermaphrodite should not be considered as simply a mixture of the two sexes but rather as the result of a definite genetic complex, analogous to that by which the pigment patterns are determined in somatic characters. It may well be that the sex-producing mechanism is cytologically unrecognizable, though present, in such cases. This is certainly true of pigment patterns.

Let us enlarge upon Wilson's suggestion for a moment, and discuss briefly the genetic basis which may lie at the root of hermaphroditism in animals.

How can hermaphroditism, the presence of male and female germ cells within the same individual, be reconciled with the idea of definite chromosomal or genetic factors for sex? Hermaphrodites in their way differ as much from dioecious forms as males and females of the latter differ from one another. Aside from the work of Schleip and Boveri, there is slight cytological evidence for the existence of any chromosome mechanism which could distribute chromosomes or allelomorphic sex factors to different parts of a single animal in the same way that the maturation divisions do in the case of bisexual forms. But it seems unreasonable to suppose that sex in hermaphrodites is a hit or miss affair, in no way related to chromosome constitution or genetic make-up, merely because chromosome differences have not been found (and in many forms probably do not exist visibly), and that presumably there is the same set of chromosomes and genes in the sperm-producing cells as there is in the ova. Like relations undoubtedly hold for other parts of animals besides those concerned with egg and sperm production.

For illustration of this fact we may discuss for a moment coat color in guinea pigs. These animals (Castle and Wright, 1916) present a large range of colors and color blends. A single animal may have black, yellow and white spots scattered over the body, and it is well established experimentally that in the guinea pig there are numerous known genetic factors which are responsible for the colors and color patterns. We must suppose that every cell in a spotted guinea pig contains the same genetic factors for hair color, yet some regions of the body are black, some white and some yellow. How can this be? The genetic and

physiological evidence shows that in guinea pigs there are genetic factors present which determine the presence or absence of certain color-producing enzymes and their distribution over the body. It is definitely known that there is a basic color-producing enzyme *a*, which, acting alone on chromogen, produces a finely granular pigment which appears yellow. It is unstable. Its intensity of production, and total absence in parts of the body is determined by a considerable number of hereditary factors (genes), some of which are definitely known. There is a second substance, *b*, which may unite with *a* to produce coarsely granular pigments which appear brown, black and sepia. Intensity of production, or absence of *b* in certain portions of the fur are determined by five definitely known and several unknown genetic factors, which give various types of color patterns.

Here we see that the genetic factor for a color can only come to full expression when acting in definite regions of the body and upon the necessary substratum, i.e., the presence of the basic color-producing enzyme.

It is permissible perhaps to draw an analogy between pigment spotting and the production of real hermaphrodites. Take the common earthworm, in one segment a testis develops, in another segment the ovaries appear. It is highly probable that every cell of the earthworm contains the same complement of genes but the full expression of the male and female determining and differentiating genes can only appear in the right internal environment. Other genetic factors determine the presence or absence of certain necessary agents (hormonal or enzymatic) in those segments in which the sex glands arise, and the germ cells thus come to rest in a different environment, genetically controlled, an environment that is absolutely essential for the expression of the sex peculiar to that segment. It is unnecessary to look for a visible difference in the chromosome constitution in spatial hermaphrodites like the earthworm, any more than it is necessary to rely upon such chromosomal differences to explain spotting in a guinea pig.

Chromosomal differences, in forms like the worm, could only be supposed to arise by somatic segregation, i.e., elimination of certain chromosomes at a definite point in the germ tract in each individual during development. No evidence for such a process exists; nor do we believe there is need for such an hypothesis.

If it is possible to assume that certain types of hermaphroditism are due to regional differentiation with respect to the substratum upon which

the sex factors interact, how is it possible to explain such astonishing cases as that described by Babor (1898) for Limax maximus? Here the individuals are first female, then hermaphrodite, then male, then again hermaphrodite and finally female. It is not improbable that in forms with maleness alternating with femaleness in the same individual, the length of the cycle and its nature are genetically determined just as other characters of the individual are determined, and are no whit more mysterious. Just as in parthenogenic reproduction the rhythms can be upset by environmental changes, so too environmental changes can lengthen and shorten the cycle of the sexes or prevent one or the other phase from developing. It is obvious that in Limex and other similar forms the hermaphrodite phase, interpolated between the male and female phases, is a period of transition, and represents the diminution of influence of one set of factors and the beginning influence of another set acting upon genotypically identical material—the sex cells.

In this connection, it is interesting to recall that in maize (Emerson, 1924) any one of nine recessive factors is sufficient, even under ordinary conditions of growth, to upset the typical hermaphrodite habit in this species. Presumably the presence of all nine of the dominant allelomorphs of these factors is necessary for the normal expression of the typical hermaphroditic condition.

Shull (1910, 1911) found that in Lychnis a single factor difference is sufficient to change a bisexual organism into an hermaphroditic one, and moreover the change is a reversible one.

To sum up, we may say that although at first sight the sex phenomena exhibited by hermaphrodites may seem to nullify all that we have said regarding chromosome combinations in relation to sex, such nullification is merely apparent and not real. That morphological chromosome differences have been found in but one or two hermaphrodites, and may not (probably do not) exist in most forms, need not disturb us greatly, for why should sex factors be presumed necessarily to influence the size and behavior of chromosomes more than do other genes? We believe that the problem of sex expression in hermaphrodites is no more mysterious or difficult of solution than the problem of the determination and differentiation of any somatic character, and it is as much dependent upon chromosomal and genetic constitution as any somatic character.

We may consider that in typical hermaphrodites the complex of genetic factors for maleness and femaleness exist in such a delicate

balance that influences similar to those which are responsible for differentiation of ordinary somatic characters bring about a more or less regular differentiation of sex organs.

It has doubtless become evident to the reader that the present day trend of zoölogical opinion regarding sex is, that in expression and inheritance it is not fundamentally different from any other character. Practically every body character is the resultant of the interaction of numerous factors although a single factor difference may greatly modify the result. Bridges (1921–1925) has advanced the view, based upon cytological and genetic data, that both sexual and non-sexual characters of an individual are the index of the point of balance in effectiveness of a large but unknown number of genes, some of which have a tendency to change development in one direction and others in the opposite. Let us consider for a moment this idea of "genic balance," and its application to sex characters.

THEORY OF GENIC BALANCE. While studying the effects of loss of one of the chromosomes of Drosophila, the so-called fourth chromosome, Bridges was struck by the fact that the phenomenon offered striking parallels to those of dioecious sex. For example, flies having but one fourth-chromosome differed in regard to many characters from flies possessing such a chromosome. The individuals were smaller in size, had smaller bristles, hatched later, were pale in body color, were less viable and had blunter wings than flies with the full chromosome complement. Each of these differences corresponded to a character for which the fourth-chromosome was internally unbalanced. So too in the case of sex, individuals with but one X-chromosome, males, present a complex of characters strikingly different from those shown by individuals with two X-chromosomes, female. Each of the differences presented by the haplo-X individual (male) corresponds to a character for which the balance of genes in the X is different from that in the group as a whole. The absence of one X leaves in action an unbalanced set of genes which produces male characters since the Y-chromosome does not carry sex factors, so far as known. Therefore X-chromosome is a chromosome that is internally unbalanced by an excess of genes that we may call female-producing. The effects of haploidy for an autosome, e.g., fourth, and the effects normally present in dioecious sex, indicate that they have a similar genic base, each being due to differences in the ratio between two aggregates of genes. The peculiarity of the X-chromosome is that it produces its characteristic effect because it

contains a preponderance of genes tending to produce the characters that we call female.

Bridges was able to produce striking evidence for his theory by study of cases in which the ratio of X-chromosomes to autosomes had been changed, and as a result of which new sex types arose. Each of the different sex types proved to be the result of a particular combination of chromosomes, and appeared in cultures of Drosophila that were triploid, i.e., that have three X-chromosomes and three of each kind of autosome. The possession of an extra X and at the same time of an extra set of autosomes does not change the individual in its sexual characters from an ordinary female. However, in oögenesis the triploid or 3n group of chromosomes is unstable, and at reduction several combinations are brought about. Normal males of Drosophila are XY in constitution, one-half the sperm carrying X, one-half Y and fertilization of the eggs of 3n females affords unique opportunity for studying the effects of peculiar chromosome combinations. Some of the individuals resulting from eggs of 3n females will have 3X-chromosomes and two sets of autosomes or be of the constitution 3X, 2A others will be 3X, 3A, or 2X, 2A. The combination 2X, 3A is especially interesting in that the zygote develops into an intersexual form, i.e., an animal that is neither male nor female, but a mixture of both sexes. In chromosome constitution the intersexes do not differ from ordinary females except in the possession of an extra set of autosomes, thus showing clearly that the autosomes are concerned in sex determination. Also they are male-determining, since the addition of a set of autosomes causes the female to assume male characteristics.

We can say, then, that in the autosomes there are genes that tend to produce male characters. Conversely the X-chromosome has a net female tendency as shown by the fact that the addition of an X to a male group of chromosomes changes the individual into a female. The net male tendency of a set of autosomes is less than the net female tendency of an X. This is indicated by the fact that in those individuals in which there are two of each, i.e., 2X, 2A, the female genes overbalance the male and the result is a female.

Bridges also found flies with the chromosome constitution X, 3A and 3X, 2A. According to theory, the former individuals should be more male than normal males, and the latter more female than normal females. The animals in fact differed decidedly from normal males and females and were termed super-males and super-females.

We now have arrived at the point where the old formulation that 2X = female is inadequate, for in the intersexes we have individuals with two X-chromosomes yet are not females. The presence of an extra set of autosomes removes them from the female class. The autosomes have an internal preponderance of male-tendency genes. According to Bridges, we may now formulate the sex-relations as follows: "Both sexes are due to the simultaneous action of two opposed sets of genes, one set tending to produce the characters called female, and the other to produce the characters called male. These two sets of genes are not equally effective, for in the complement as a whole, the female-tendency genes outweigh the male-tendency genes and the diploid (or triploid) form is a female. When the relative number of the female tendency genes is lowered by the absence of one X, the male-tendency genes outweighs the female and the result is the normal haplo-X male. When the two sets of genes are acting in a ratio between these two extremes, as is the case in the ratio of 2X:3A, the result is a sex intermediate an intersex."

In a recent paper, L. V. Morgan (1925) has furnished an interesting confirmation of Bridge's theory of intersexes in Drosophila. She studied a strain of flies in which the two X-chromosomes were permanently attached (XX-linkage). Intersexes arose from certain matings, and cytological examination showed that these individuals had the chromosome constitution, XX, 3A and 2X, 3A.

The "genic balance" interpretation of sex, namely, that sex is probably an expression of the interaction of several, probably many, factors located in different chromosomes has received strong support from the work of Emerson (1924) on maize.

The normal plants of this species are hermaphrodites; the termial inflorescences being male, the lateral inflorescences, female. Certain types of maize are known to have occurred as mutations which are entirely female. Four distinct recessive genes are known, the influence of any one of which results in femaleness. Maize which is altogether male is unknown, but there are three types which produce but a few pistillate flowers. Each of these three types is known to be due to one or more recessive factors, each transmitted independently of the other. Emerson also cites cases of two types of maize in which the monoecious habit has been modified to an andromonoecious one, and he states that each of these two types is also due to the influence of one or other of two recessive genes. From this account, it seems that in maize there are nine (probably many more) pairs of genetic factors which influence

the expression of sex, and several of these are known to be borne on different chromosomes.

Sturtevant (1921) has described a race of intersexual Drosophila simulans. The intersexes are modified females and they possess two X-chromosomes even in the male-appearing parts. The modification of the female is produced by a recessive mutant gene located in an autosome. This case shows clearly that the sex-determining mechanism, despite the fact that the reaction had proceeded in a definite direction—female, can be thrown out of gear, so to speak, or greatly modified by the action of an autosomal gene. It is evident, from the works cited, that sex expression is the result of many genes, and not simply of an allelomorphic pair.

So far, the discussion has centered around chromosomes and the Mendelian factors borne by them, and has been colored more or less by morphological considerations. However, the topics discussed in the next section require for explanation formulation in physiological terms. The study of intersexes and sex reversal has rendered it imperative that some of our morphological concepts of sex determination be supplemented by physiological ideas.

INTERSEXES AND SEX REVERSAL IN LYMANTRIA. The best known case of intersexuality and sex reversal is that of the gypsy moth, Lymantria, studied by Goldschmidt (1912–1924). This insect has a wide geographical distribution, and the sexes are well marked off from one another in many structural features. When moths from widely different localities are crossed the progeny exhibit peculiar sexual abnormalities. Thus, crosses of male moths from a Japanese race, with females from certain European races, give normal males, but the females are a mixture of male and female characters. They are called intersexes. Further investigation showed that many different races of Lymantria exist which differ in regard to the factor or factors responsible for the production of intersexuality. This is so, because the extent of the assumption of the characters of the opposite sex is definite and typical for a given cross. Males and females of the same race always give normal offspring, but when the male or female of one race is bred with individuals of various other races, all kinds and degrees of intersexuality result—even complete sex reversal. By making appropriate crosses the sexes can be reversed, and the actual process of reversal studied embryologically.

Goldschmidt accepts the chromosome theory of sex and applied it to his Lymantria material in an ingenious manner. It will be recalled that the progeny of all races employed in the crosses are quite normal

when males and females of the same race are used. In the right combination the eggs and sperm give only normal sex results; in hybrid combination, however, the same cells give rise to intersexual individuals. The result seems to depend upon a relationship of something in the eggs to something in the sperm. This something in hybrid combination is quite different from the normal. Consequently, a definite quantitative relation exists between the two sorts of sex-determining substance which is normal, any other quantitative relation produces intersexuality. The degree of intersexuality thus depends upon the degree of variation of such quantitative relations from the normal. According to Goldschmidt, each sex contains the factors for both sexes, and which actually does develop depends entirely on the quantitative relationship between the two factors. For illustration, let F denote the factor for femaleness and M the factor for maleness, then the formula for the two sexes will be (F) Mm = female; (F) MM = male. Goldschmidt places brackets around the F to indicate that it is purely maternal in inheritance. The female is heterozygotic in the factor for maleness, which is carried by the X-chromosome. The factor for femaleness carried in the cytoplasm or W-chromosome is handed on equally to every egg, and is purely maternal. The factor F and M act independently with a quantitatively determined power which may be termed the valency. The factor with the greatest valency determines the result. The quantities are, however, not equal so that one M is weaker than one F, thus in the female sex when both are present the M produces no effect. Two M's, however, are stronger than one F and consequently in the male the F is impotent.

Goldschmidt has formulated some interesting physiological ideas regarding the nature of the sex-determining substances and as to how the quantitative effect is to be interpreted. He identifies the sex factors distributed by the chromosome mechanism with enzymes, and the end result of their activity is the production of hormones which control sex differentiation. Thus, according to Goldschmidt, the provisional solution of the problem of intersexuality in Lymantria is as follows: Each fertilized egg possesses male or female determining factors. These factors are enzymes and each is necessary in order to produce the hormones of sex differentiation. In forms exhibiting female digamety, like Lymantria, every egg is identical in regard to the factor for femaleness. In the male, the sex factor is carried by the X-chromosome and it will be present in half of the eggs and in all sperm. The mechanism of sex inheritance which consists in the distribution of two X-chromosomes

to the male (female digamety) and one X to the female, is a mechanism which decides at the commencement of development whether n or two n-units of male enzyme will be oppossed to a definite and always equal quantity of female enzyme. These quantities are so graded that the amount of female enzyme is greater than the n of the male enzyme, and so the production of hormones of female differentiation is accelerated in this combination and the development is female. On the other hand, two n of male enzyme is a higher concentration than q of the female, consequently the hormones of male differentiation will be produced more rapidly and a male will result.

Races of moths vary in regard to the absolute quantity of the enzymes and if two such races are crossed which differ in this respect the normal quantitative relation is destroyed, and male enzymes even in one n quantity may be too strong for the female quantity. The result would be an individual which begins development as a female but as a result of the abnormal quantitative relation of the sex enzymes the hormones of sex differentiation are formed too rapidly, an effective amount is accumulated at some point in embryonic differentiation, and the sex reaction is reversed, the individual ending up as a male or markedly intersexual moth.

As an outgrowth of Goldschmidt's experimental work, zoölogists now recognize two types of intersexuality: zygotic and hormonic, the former occurring in insects, the latter in vertebrates, especially birds and mammals. In insects, each cell of the individual possesses all that is necessary for its determination, and thus each is independent of the remainder of the organism. In other words, sex characters and sex differentiation in this group are not dependent upon the presence of a sex gland. This is a well-established fact as shown by castration and transplantation experiments (Oudemans, 1899; Kellog, 1904; Regan, 1909; Meisenheimer, 1919; Kopec, 1913; Steche, 1912; Geyer, 1913, and Kornhauser, 1919). On the other hand, in birds and mammals (Tandler and Grosz, 1913; Lillie, 1917; Lipschutz, 1914; Sand, 1918; Moore, 1919; Steinach, 1913–1920) the sex factors distributed by the chromosomes at fertilization carry sex differentiation only a short way, so to speak, to the point where a definite sex gland is formed. From this time on, the gonad secretes sex differentiating hormones that are essential for complete sex expression. This is also a well-established fact. In the insect, the hormones are produced in every cell of the body, but in birds and mammals the secretions are localized in one central organ—the sex gland. Thus

it is that one can remove the sex gland of larval moths without effect upon the sex characters, whereas in birds and mammals gonad removal in early developmental stages leads to profound alteration of the sex characters—indeed, in birds actual sex reversal may result.

SEX REVERSAL IN VERTEBRATES. During the past few years several apparently authentic cases of complete sex reversal in vertebrate animals have been reported. Such cases are of considerable importance for sex theory and need to be carefully examined. We shall discuss briefly the best known cases.

It will be recalled that the genetic evidence shows that the chromosome constitution of birds is the WZ-ZZ type, the female producing two kinds of germ cells, whereas the spermatozoa are all alike. Under normal conditions, the sex of these animals is established at fertilization. However, some recent evidence indicates that the type of sex organization and reproductive functioning of birds is not in all cases irrevocably determined by the sex chromosome constitution, as the following case indicates.

Crew (1923) obtained from the owner a three and one-half year old Buff Orpington hen. The owner claimed that the hen previously laid eggs and had hatched young from them before ceasing to lay at about 3 years. According to Crew, the hen first seen by him was normal in appearance, but was developing a tendency to crow. During the next twenty-two months the hen assumed the plumage, copulatory and fighting behavior of a normal cock, and in fact functioned as a normal male by fertilizing two eggs of a virgin Buff Orpington hen and became the father of two young—a male and a female. After the bird died autopsy revealed extensive tuberculous lesions of the viscera. Two normal-appearing testes were found, and also the degenerate remains of an ovary. Riddle (1924) has described a somewhat similar case in pigeons.

The two cases just mentioned involved sex transformation of adult animals. The following case was experimentally induced. Benoit (1923) discovered that following removal of the left ovary of young fowls (the right ovary normally degenerates in embryonic life), the birds assume the characters of normal cocks and the assumption of these characters is correlated with the development of a structure resembling a testis on the site of the degenerate right ovary. Living sperm cells were found in the birds. Previously, Zawadowsky (1922) and Goodale (1916) had noted the formation of a peculiar testis-like body on the right side following ovariotomy, but they failed to recognize its significance.

Benoit regarded his work as demonstrating that female birds are really suppressed hermaphrodites, i.e., the left side is female and produces an ovary, the right side is male and is capable of producing a testis but this is suppressed in development by hormones emanating from the left ovary. However, in the light of Crew's and Riddle's cases, and from the fact, that the right ovary occasionally persists in birds (Riddle, 1925), it seems pretty clear that Benoit really demonstrated that the sex of birds can be reversed experimentally (Domn, 1924). If we turn from birds to some of the lower vertebrates we find one or two similar if less striking facts. Sex conditions are peculiar in frogs and toads (Hertwig, 1912; Swingle, 1920, 1922, 1925; Witschi, 1914, 1920, 1922). In 1912 Meyns published an account of gonad transplantation in young frogs. He transplanted pieces of the testis of one animal under the skin or into the peritoneal cavity of another. Such grafts undergo considerable degeneration. However, if the transplants were left undisturbed for some months, regenerative processes set in and the germ cells (spermatogonia) developed into oöcyte-like, or egg-like cells, indistinguishable from immature ova. Meyns concluded that the ova-like cells were really ova, and had arisen from the male determined germ cells.

A similar but more striking case is that recently reported by Guyénot and Ponse (1923–1924). They observed that small pieces of the testis of toads when transplanted under the skin of another animal, or in an abnormal position in the same individual, underwent an initial degeneration. Later a regenerative process followed, in which both sperm and egg-like cells developed side by side from sister cells of the same graft. They regard the egg-like cells as immature ova. These early undergo degeneration.

Now, just how are we to regard these cases of apparent sex-reversal in vertebrates? It seems probable that genotypically the cells of the right and left sex glands of embryo fowls are alike, and if we accept the opinion of Meyns and of Guyénot and Ponse with regard to the oöcyte-like cells described by them, then there can be no question but that genotypically identical sex cells, existing side by side are capable of differentiating along male and female lines.

If we assume, as is frequently done by some writers, that maleness and femaleness stand in the relation of simple allelomorphs associated with a single pair of factors, and consequently that the two sexual states are mutually exclusive, we find that hermaphroditism and sex-reversal are utterly inexplicable. However, according to the newer and saner view,

the two sexual states appear to result from the interaction of many factor pairs, which severally exist in the allelomorphic condition. The entire complex of genes with male and female tendencies is balanced in a sexually normal individual, and changes in the sexual state (e.g., sex reversal) result from shifting of the balance in a plus or minus direction. It is perfectly obvious that each sex contains the anlagen of the characters of the opposite sex. These are normally not expressed in dioecious organisms because the balance is such that only one of the two sexual states becomes manifest. However, it is conceivable that some radical change in the internal or external environment of the organism, or developing cell, might overthrow the balance of the sex factors established at fertilization and swing the sex in the reverse direction.

Take the case of birds: here we have excellent genetic evidence that under normal circumstances sex in these animals is determined by the chromosome constitution (i.e., the chromosome carries the genetic factors). The complex of genetic factors distributed by the chromosome mechanism is so balanced that one or other of the sexual states is expressed, the other remaining latent. In this group of vertebrates we find also that the sex glands with their hormones are the agents through which the genetic factors work in bringing one or the other sex to full expression. The primary sex-determining agent is the balance of genetic factors as represented by chromosome constitution. These factors give the initial push to sex determination and differentiation, and they are early reinforced by hormones from the gonads which accelerate the process of differentiation. The hormones are the result of other genetic factors and they serve to inhibit definitely the bisexual potentialities of the individual. The evidence from castration and transplantation experiments indicates that the original "genic balance" which served to swing sex definitely one way or the other, is quickly reinforced by accessory sex differentiating agents—the hormones.[2]

Thus we can see how it is possible for a testis-like structure to develop on the right side when the ovary is removed from some birds. It would appear, in such cases, that removal of the inhibiting agent (ovary) tends

[2] It is necessary to bear in mind the distinction between sex determination and sex differentiation. In insects the two are much the same, but in vertebrates the zygotic sex-determining factors are limited in their capacities to bring about sex differentiation, and are reinforced by hormones from the sex glands which regulate and control the differentiation processes. By appropriate manipulation of the hormones (extirpation and transplantation) the differentiation process may be reversed in a sexually determined individual.

to overthrow the original "genic balance" established at fertilization so that the latent potentialities of the opposite sex are able to express themselves. So we arrive at the conclusion that sex expression, like the expression of any somatic character, is the result of the interaction of many genes carried in the various chromosomes. It is the expression of a balance struck between the opposing tendencies on the part of the organism. What is really inherited in the matter of sex, as Emerson (1924) phrases it, is "the necessity of reacting in a particular way to a particular internal and external environment." Occasionally violent changes in the internal environment may possibly upset the sex balance and the reaction go in the reverse direction. This does not mean that the sex factors or chromosome constitution themselves change. Far from it, for it is well established experimentally (Morgan, Emerson, etc.) that genetic factors may vary greatly in as far as the effects which they produce are concerned, yet despite the fact the expression of the resulting character is greatly altered, the genetic factor itself remains unchanged. So too with sex reversal, the "genic balance" is upset, the sex reaction reversed, but not a single factor or chromosome is changed thereby, but only the relation in which they stand to one another as parts of this interacting system is altered.

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THE PITUITARY BODY

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The enormous literature on the pituitary body is such as to render an adequate discussion of our knowledge on all phases of this gland entirely beyond the scope of this review. Hence only selected topics dealing with the more recent researches will be discussed. Fortunately, there are numerous excellent and comprehensive works on the hypophysis, which deal with aspects of the subject that are not herein considered, to name only a few such as *Endocrinology and Metabolism*, edited by Barker, Hoskins and Palmer; *The Pituitary and its Disorders*, by Cushing; Biedl's *Innere Sekretion*, Swale Vincent's *The Internal Secretions*, Blair Bell's *The Pituitary*, Houssay's *La acción fisiológica de los extractos hipofisiarios*, Schafer's *The Endocrine Organs*, and numerous comprehensive discussions in papers to which reference will be made in the text, and the reader can therefrom supplement the shortcomings of this review. In the present report, account will be taken only of the experimental work which can be properly regarded as covering the modern development of our knowledge concerning the pituitary body. The origin of the word pituitary and some of the early views about the physiological rôle of this gland and some of the significant events in the development of our modern conceptions of the endocrine organs are admirably set forth in a recent Harvey Lecture by Abel, to which frequent reference will be made.

The first experiments to demonstrate that the pituitary gland contains a substance possessed of striking physiological properties were carried out by Oliver and Schafer in 1894. These investigators discovered that when extracts of the pituitary gland are injected intravenously into an anesthetized animal there occurs a rise of blood pressure which comes on more slowly but is more prolonged than occurs after the intravenous injection of extracts of the suprarenal glands. These authors further report a diminution in frequency but an augmentation of the force of the heart beat after injections of pituitary extracts.

Szymonowicz, working about the time of Oliver and Schafer, reported as a result of two experiments with pituitary extract, a slight lowering of the blood pressure and an increased heart heat. He either did not extract the principle from the gland, or injured it, thereby destroying its pressor activity.

Howell in 1898 showed that this pressor principle is found in the posterior lobe of the gland. He further called attention to the fact that subsequent injections of infundibular extracts either caused a smaller or no rise in the blood pressure. To quote Howell's own words: "It would seem that an injection of this extract made directly into the circulation leaves the heart and blood-vessels in a condition of diminished irritability toward a new injection. The blood pressure and pulse rate in such cases simply return more or less completely to the normal, and new injections have either a negative result entirely or an effect more temporary than that of the preceding injection."

It will be shown later that this tolerance, as well as an actual inversion of the blood pressure level after repeated injections of the posterior pituitary principle, is not due to the presence of depressor substances but is an inherent property of the principle which, when freed from depressor and other foreign bodies, possesses in a striking degree and in the highest dilution all the physiological properties of a well prepared extract of the gland.

A few years before Oliver and Schafer's work, researches were already being done on the effects of the removal of the pituitary body. Sir Victor Horsley, in 1886, reported the removal of the hypophysis from two dogs that lived five and six months after the operation and without any apparent untoward effects.

About this time also, clinicians began to correlate pathological conditions with disturbances in the function of the pituitary gland. Marie, in 1886, published a clinical description of two cases of acromegaly and erroneously attributed them to a hypofunction of the pituitary gland. Minkowski in 1887 suggested that the hyperplasia of the pituitary gland in acromegaly is a causative factor in the disease. The subsequent development of the subject will be traced in a later section under Extirpation Experiments.

THE ANATOMY OF THE PITUITARY BODY. Space permits of only the briefest mention of the constituent parts of the gland which in man and the higher animals occupies the fossa hypophyseos of the sphenoid bone in the floor of the cranium. There are certain structural variations in the gland of different species of animals; in the lower species

the different parts of the gland are distinct and easily separable but in the higher forms the different lobes are closely united and less easily separable. In man and the higher animals the hypophysis cerebri is made up of three lobes—anterior, intermediate and posterior—which are attached to the brain by means of a stalk or infundibulum. The pars anterior is the largest lobe, glandular in structure, and has a rich blood supply. The hormone which exerts a powerful influence on growth functions comes from this lobe. The pars intermedia, or juxtaneural epithelium, the smallest portion of the gland, closely invests the pars nervosa or posterior lobe, and extends up the stalk. Its extent and distribution vary greatly in the different species of animals. It is composed of secreting cells, some of which dip in strands into the substance of the posterior lobe, which is made up largely of neuroglia tissue poorly supplied with blood and lies upon and is partly enveloped by the anterior lobe. The "pars tuberalis" (Tilney), a third epithelial lobe, extends nasalwards from the junction of the pars intermedia and the anterior lobe to surround the infundibulum and to spread out for a variable distance under the brain floor. There exists also a pharyngeal hypophysis.

The separate divisions of the gland have a different embryological origin; the anterior lobe is derived from the ectodermic wall of the buccal cavity, the posterior lobe from the ectoderm of the thalamencephalon (Herring). The weight of the average normal human hypophysis is 0.6 gram according to Erdheim (see also Rasmussen and Saito).

The origin of the secretion generally designated as the "posterior lobe" hormone is an unsettled question. Many investigators regard the hormone as being secreted by the cells of the pars intermedia and stored in the posterior lobe from which it is ordinarily extracted (Blair Bell, Houssay, Atwell and Marinus). Maurer and Lewis find a true secretion antecedent in the pars intermedia upon histological evidence only. In the stalk or infundibulum, as well as in the floor of the third ventricle, is found hypophyseal functioning tissue (posterior lobe hormone). The significance of this will be discussed more fully when an attempt is made to reconcile the divergent views regarding the polyuria and other disturbances induced in animals with intact hypophysis merely by injury to the floor of the third ventricle, or to explain the lack of symptoms following hypophysectomy.

At this time it may be of interest to mention that the mode of exit of the posterior lobe hormone is also still problematical, that is to say, uncertainty exists as to whether the principle is taken up directly by

the blood stream or leaves by way of the cerebro-spinal fluid. Herring in 1908 first suggested that the posterior lobe secretion passes into the cerebro-spinal fluid. His suggestion came as a result of finding in the nervous portion of the posterior lobe large quantities of colloid material which he thought represent products of secretory activity of the cells from the pars intermedia, and which pass through the tissue interstices of the pars nervosa into the third ventricle.

Cushing and Goetsch confirmed the histological finding of Herring, and also sought proof of the presence of the posterior lobe hormone in the cerebro-spinal fluid. They, accordingly, concentrated spinal fluid obtained from patients, injected it into dogs and rabbits and found a distinct rise of blood pressure following the injections, thus indicating the presence of a pressor body in their concentrated material. Jacobson (1922) obtained depressor effects when concentrated cerebro-spinal fluid was injected into anesthetized dogs. Bauer obtained by intraperitoneal injections of human cerebro-spinal fluid falls in the temperature like those produced by pituglandol.

Carlson and Martin raised an objection to Cushing and Goetsch's work, stating that the presence of inorganic salts in the concentrated cerebro-spinal fluid may have a pressor action; they injected unconcentrated cerebro-spinal fluid and obtained no pressor response. Wassing could not demonstrate the presence of vaso-constricting substances in the cerebro-spinal fluid by using the frog perfusion method. Houssay obtained cerebro-spinal fluid from various sources, concentrated it, injected it into dogs and rabbits and obtained a pressor response exactly like that obtained by Cushing and Goetsch. He also obtained increased contractions of the bladder, stomach and intestines with this concentrated material. Furthermore, with 5 drops of diluted cerebro-spinal fluid he obtained mydriasis in the enucleated eye of a frog, and contractions of the isolated esophagus of the frog. These experiments might seem to indicate that the post-pituitary principle is present in the cerebro-spinal fluid. Houssay, however, found that unlike the posterior lobe hormone, treatment of the cerebro-spinal fluid with lead acetate followed by hydrogen sulphide or sulphuric acid resulted in complete loss of the pressor principle; in fact he secured a strong depressor substance. Houssay, recognizing the fact that long heating of the liquid in both his and Cushing and Goetsch's experiments, might have weakened or destroyed the principle, tested the action of the unheated liquid on the mammary gland—a method which he claims is capable of detecting exceedingly minute

amounts of posterior lobe hormone—and found that even as large an amount as 50 cc. of liquid has no galactogogic action whatever. From these and other facts Houssay concludes that the pressor principle in the cerebro-spinal fluid differs from that found in the posterior lobe. Weed and Cushing find that the injection of posterior lobe extracts causes an increased secretion of cerebro-spinal fluid as measured by inserting a graduated and calibrated needle into the third ventricle of a dog. Dixon tested the action of cerebro-spinal fluid of dogs on the isolated uterus of a virgin guinea pig, and obtained a marked contraction, thus demonstrating the presence of a substance possessed of oxytocic properties in the cerebro-spinal fluid. Dixon and Marshall have recently demonstrated that in experimental animals the cerebro-spinal fluid contains an increasing amount of oxytocic material in the later stages of pregnancy. Trendelenburg and Miura working independently arrive at the same conclusion as Dixon, but in addition find that after removal of the hypophysis from dogs no such oxytocic substance can be detected in the cerebro-spinal fluid. The finding by Atwell and Marinus that an extract of the "pure neural stalk" contains a powerful pressor body (presumably post-pituitary hormone) adds further support to the idea that the secretion of the pars intermedia passes into the neural lobe and then through the neural stalk into the third ventricle. Certain earlier authors have reported negative results in testing for the presence of the post-pituitary hormone in the spinal fluid. Among these may be mentioned C. M. Oehme and Leschke.

The evidence brought forth by Cushing and Goetsch, Dixon, Trendelenburg and Miura and Atweil and Marinus, while quite suggestive, is not conclusive as yet.

In a paper by Maurer and Lewis the view that the posterior lobe secretion of the pituitary escapes by way of the cerebro-spinal fluid is submitted to a critical examination. (When they wrote their paper the researches of Dixon, Miura and Trendelenburg were not published.) They produce evidence for the first time of a true secretion antecedent in the cells of the pars intermedia, "an antecedent which appears in the cells at the same period of development at which pressor effects may be obtained from the gland extracts." Using pig's embryos, Lewis finds that extracts of the hypophysis of a 7.5 cm. pig embryo have no pressor activity and no granules, but from the pituitaries of 17.5 cm. pig embryos the pressor substance is obtainable. At this period for the first time the granules or secretory antecedent of the pars intermedia are discernible. In discussing the route by which the secretion

leaves the gland, they say that the fetal pig's hypophysis contains no hyaline bodies (the material from which the posterior lobe secretion is derived, according to Herring, and Cushing and Goetsch). Maurer and Lewis say: "Indeed they are rare in the adult, though there may be seen in the cells of the pars nervosa in the processes of its intrinsic cells, granular deposits which we believe to be the antecedents of the hyaline bodies but which in the pig rarely are discharged and aggregated into discrete masses as in other mammals." These investigators are inclined to the view that the secretion leaves the gland by the vascular route rather than by way of the interfibrillar spaces of the pars nervosa. Blair Bell, too, believes in the direct vascular route as the avenue of escape for the hormone formed in the pars intermedia.

Probably the final proof will only be forthcoming when we have a delicate, specific chemical test for the posterior lobe hormone which, if present in the cerebro-spinal fluid, must be there in the minutest concentration. Although technical difficulties are involved, it would no doubt be possible to take large amounts of cerebro-spinal fluid, free it from inert material such as the inorganic salts and protein material, and then test the residue for the multiple physiological properties (pressor-oxytocic-diuretic-antidiuretic) which, as will be shown later, are all vested in the post-pituitary hormone. And should this residue, freed from other material of the spinal fluid, be shown to have the above-named physiological properties, then one may be fairly safe in concluding that it is identical with the hormone of the posterior lobe, and that the mode of escape of this hormone has been settled.

THE ACTIVE PRINCIPLES OR HORMONES IN THE PITUITARY BODY. In discussing this phase of the subject it is essential to take up the anterior and the posterior lobes separately for the reason that in each we find a principle or principles which have different physiological functions, although a certain amount of overlapping will inevitably occur.

THE ANTERIOR LOBE. Evans in a recent Harvey Lecture[1] comprehen-

[1] Although this lecture has not been published, the writer was privileged to receive a copy of the manuscript from Professor Evans about the time this review was ready for the press. Reference in the text to the data of Evans and coworkers was secured from published communications. The main facts reported in the preliminary papers have been substantiated by long continued experiments with large numbers of animals. In addition further chemical studies have been made which point definitely to the presence of two hormones in the anterior lobe, one which promotes growth and the other which retards ovulation. Alcohol in concentrations between 5 and 50 per cent apparently precipitates the growth principle, but not that which inhibits ovulation. By suitably adjusting the pH the material obtained from the anterior lobe can be freed of a large amount of protein by centrifugation without affecting its potency.

sively reviews our knowledge of the anterior lobe of the hypophysis. The fact of the presence of growth-stimulating and ovulation-inhibiting hormones in the pars anterior of the pituitary body seems to be established with certainty. (A comment may be interjected here: although the existence of the hormone is definitely known very little work has been done on the chemical properties and nature of this principle; the only work on this phase is that of Robertson, Smith and Cheney, Drummond and Cannan and Evans and co-workers.)

The experimental data which form the basis for the belief in the presence of a growth-stimulating hormone were obtained by several well-known procedures, namely:

1. Feeding the whole gland, or pars anterior to normal or hypophysectomized animals.
2. Extirpating the whole gland, or the anterior lobe alone.
3. Transplanting the gland into normal or hypophysectomized animals.

Feeding and Injection Experiments. The majority of investigators (Caselli, Sandri, Schafer, Aldrich, Cushing and associates, Lewis and Miller, Klinger, Pearl and Surface, Sisson and Broyles, Hoskins, Hogben, Evans and Long, Drummond and Cannan, C. S. Smith, Wulzen and Patterson) report either no influence or even a retardation of the growth or development of experimental animals (dogs, guinea pigs, rats, mice, fowls, tadpoles and salamanders, flesh flies) when whole pituitary gland or anterior lobe alone is fed. On the other hand, Robertson, Goetsch, Marinus and Dott state that feeding anterior lobe tissue to dogs, cats, rats and white mice has a stimulating action on their growth and their reproduction apparatus. Similarly Uhlenhuth, Smith and Cheney, Hoskins and Hoskins claim some stimulation of body growth from feeding anterior lobe of the pituitary body to metamorphosed salamanders and to amphibian larvae.

The lack of concordance in the results obtained by feeding the anterior lobe of the whole hypophysis to experimental animals is not surprising when one considers the many factors which have to be taken into account in experiments of this type. To mention but a few: variations in animals of the same species; use of different species in which the response may be different; variation in the mode of preparation of the material used (the principle may be injured or even destroyed before being fed); failure of absorption of the hormone; or its destruction by the gastro-intestinal enzymes or by bacteria in the large intestine.

Were we to rely on feeding experiments only for the proof of the presence of a specific growth-stimulating hormone in the anterior lobe of the hypophysis, then we might reasonably entertain some doubt as to the soundness of the evidence adduced, owing to the number of experiments in which no positive results were obtained. Several investigators (Evans and Long, Smith, and Hogben) have recently called special attention to the fact that in their experiments no growth-stimulating effect was observed when anterior lobe preparations were fed to their experimental subjects (rats, tadpoles and Axoloth larvae of Amblystoma tigrinum), whereas most definite changes resulted when similar preparations were injected. Due to the fact that these experiments are so strikingly and so well controlled much importance must be attached to them notwithstanding the fact that earlier investigators (Caselli, Cerletti, Crowe, Cushing and Homans, and Pearl and Surface), working with different species of animals (mice, guinea pigs, puppies, dogs and fowls), were unable to produce any signs of hyperpituitarism by injecting either whole gland or anterior lobe pituitary extracts. Crowe, Cushing and Homans ascribe the loss of weight in their puppies and adult dogs following the injection of whole gland extracts to the inclusion of the posterior lobe, because no such loss of weight resulted from the injection of anterior lobe extracts alone. The same explanation probably holds for the retardation in growth of some of Caselli's and Cerletti's animals, and is no doubt applicable as well to Foderà and Pittau's experiments in which emaciation followed from the injection of pituitary extracts.

Pearl and Surface, in connection with their studies on the physiology of reproduction in the domestic fowl, were interested to ascertain whether there is a chemical substance which would activate the resting ovary. The anterior lobe of the pituitary gland of the cow was considered as likely to contain such a "chemical activator," and accordingly extracts of this substance were injected intraperitoneally into hens in which the ovary was in a completely resting condition. The results were entirely negative in that ovulation was not induced any earlier than it would normally occur. Clark, about the same time, fed the anterior lobe of the hypophysis taken from growing mammals and found a definite increase in the egg-laying capacity of hens; the hatchability of their eggs was also increased. The investigations of Pearl and Surface were continued and extended by Pearl who found that feeding the anterior lobe to hens during a period of low fecundity did not increase their egg-laying capacity. Furthermore

feeding the same substance to growing pullets did not hasten the activation of the ovary; and the growth of chickens was definitely retarded by the feeding of the same anterior lobe material. Simpson has recently confirmed Pearl's finding. Walker (cited by Evans) has arrested and indefinitely prevented ovulation in hens by the administration of anterior lobe extracts.

Owing to the clear-cut nature of the investigations of Evans and Long, it may be of interest to give the particulars of one of their experiments which was carried on for 333 days, at the end of which time a rat receiving intraperitoneally anterior hypophyseal substance weighed 596 grams and its healthy litter mate control weighed only 248 grams. These investigators write as follows about this experiment.

> It would not appear to be incorrect to characterize these changes as producing constantly a certain degree of true gigantism. Increase in weight results to a great extent from a storage of fat, but is not solely due to this, the skeleton being invariably somewhat larger and heavier, and, as would be expected, the heart, lung, alimentary canal and kidney are heavier. The fat deposits in the omentum and mesentery are such that these structures weigh three or four times as much in the experimental animals as in their controls. The weight of the hypophysis, thyroid and thymus is not appreciably affected.
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> Most surprising is the effect on the reproductive system. *Oestrus as detected by changes in the vaginal smear, may never occur in these animals or may be exhibited only at long intervals.* It was, consequently, surprising to find that in all instances the ovaries instead of being underdeveloped weighed twice as much as they did in the control animals and exhibited great numbers of substantial corpora lutea. The uterus, on the contrary, weighed absolutely about half as much as it did in the normal controls. Histological examination of the gonads confirmed the presence of very abundant lutein tissue and demonstrated the formation of this tissue about the egg in unruptured normal follicles and in atretic follicles. Ripe, normal Graafian follicles were invariably absent. *A powerful, specific stimulus to lutein cell transformation has thus been effected by this hormone.*

Subsequent experiments with Flower and others have revealed the presence of two separable hormones in the anterior lobe—one which stimulates growth and the other which inhibits ovulation. Alcohol is used as the differential precipitant.

Controls with fresh posterior lobe substance showed none of the above changes.

Hogben writes that "while pituitary feeding has no influence on the metamorphosis of medium-sized sexually mature Axoloth larvae of Amblystoma tigrinum injection of anterior lobe extracts into Axoloths of the same ages and dimensions was followed by the assumption of

the adult characteristics with a rapidity comparable to metamorphosis induced by thyroid administration." Spaul injected commercial extracts of pars anterior and was successful in stimulating growth in one case in the Mexican salamander; the melanophore response was in all cases tested on this species.

Very little work has been done to ascertain the chemical nature and properties of the active principle of the anterior lobe of the hypophysis. Robertson has obtained from the pars anterior of ox pituitaries by means of boiling alcohol and subsequent precipitation with dry ether a fraction named by him "tethelin." This product, which no doubt is a mixture of substances, is claimed by him to have all the characteristic actions (marked retardation of the first portion of the third growth cycle followed by acceleration of the latter portion of the third cycle; and a prolongation of the normal life-span; Robertson and Ray) of the anterior lobe of the hypophysis when tested on the growth of white mice by feeding it to them. P. E. Smith, working with hypophysectomized tadpoles, reports no influence on their growth by feeding tethelin. He finds that the growth-promoting substance is contained in the residual alcohol-insoluble portion of the anterior lobe. Robertson in a rejoinder ascribes Smith's negative results to the fact that the tethelin was added to the water in a concentration insufficient to affect the growth of the hypophysectomized tadpoles. Drummond and Cannan were unable to substantiate Robertson's findings. They state that tethelin is a very impure mixture of substances of the lipoid class. Robertson has replied to the criticisms of Drummond and Cannan.

Iodine has been found in the anterior lobe in very small amounts by Schnitzler and Ewald and by Hoskins and Hoskins. The studies of Halliburton, Candler and Sikes and Wells and Seaman do not substantiate these findings. Simpson and Hunter did not find any increase in the iodine content of sheep's pituitary glands six months after thyroidectomy. This fact was used as an argument against the view of an hypophyseal compensatory hypertrophy following thyroidectomy.

Smith and Cheney, using tadpoles, tested fresh anterior lobe substance and dried glandular tablets obtained from several sources, and found that the tablets having a higher iodine content altered decidedly the development of the normal tadpole, while the product low in iodine had no influence on development. Addition of potassium iodide and thyroxin to the products low in iodine had a beneficial effect. The

possible influence of the iodine in the thyroid may have to be considered here.

Whether iodine belongs to the molecule of the active principle of the anterior lobe or not can only be settled when the hormone has been prepared in purer form than has hitherto been the case. Is it not possible also that the gland may harbor an iodine-containing non-specific principle in the case of animals living in water or regions where iodine is relatively more plentiful?

Smith and Smith find that the only principle which appears to retain its activity under all of the treatments (boiling with distilled water or 0.3 per cent hydrochloric acid) to which they subjected the anterior lobe substance is the principle evoking a reaction from the pigmentary system.

No endocrine nor growth responses were given by the anterior lobe after these treatments. Treatment with alcohol, on the other hand, does not appear to destroy the endocrine principle.

Further comments on feeding and injection experiments with endocrine principles. For several reasons a certain amount of conservatism is necessary in drawing conclusions from experiments in which glandular extracts are fed or injected: 1, the amount of the active principle present in such extracts must be very minute indeed; 2, the active principle is admixed with a great deal of other material, such as salts, lipoids, proteoses and proteins, and the introduction of some of these may exercise a pronounced effect on the animal, and even act antagonistically to the active principle, or be sufficient to mask or alter the true action of the hormone. Furthermore, 3, the injection of the hormone, admixed with foreign matter, is very different from the process which goes on in the body, where the hormone is taken up continuously by the blood-stream and distributed. 4, Hormones appear to be unstable substances which may be lost during the manipulations attendant on their extraction or they may be injured and rendered less potent during these processes. There are, perhaps, other factors which have not been mentioned, but it seems safe to say that the injection into an animal of a principle, perhaps admixed with foreign substances, some of which exert a marked influence in themselves, is an entirely different matter from the natural processes which go on in the body whereby the supply is continuous and not mixed with foreign materials. Of course, the purer the preparation, the more nearly, it would seem, one can approach nature's method of furnishing the body's regulators. Even the introduction of the chemically pure

principle is, however, but an imperfect attempt at reproducing the mechanism at work in the production of the disease conditions now believed to be associated with a hyperfunction of a particular gland. Finally, if some of these endocrine principles have *regulatory* functions within the animal economy, and if we subscribe to the ideas so well discussed in a lecture by Meltzer on "The Factors of Safety in Animal Structure and Animal Economy," then one may easily conceive of circumstances when any surplus of a hormone in the normal animal may be suitably disposed of without setting up a pathological state of hyperfunction with its attendant structural or metabolic changes and such was probably the case where experimenters failed to obtain positive results with feeding or injection experiments. When, however, such striking and conclusive results as those of Evans and Long and co-workers, and others are obtained the proof of endocrine overfunction is definitely established.

Extirpation Experiments. The great mass of literature on this topic has been extensively reviewed by several investigators, such as Paulesco, Crowe, Cushing and Homans, Aschner, Bremer, Dott and more recently by Dandy and Reichert, hence only a brief summary will be given here.

Marie's clinical description of acromegaly and Minkowski's association of this disease entity with a disturbed function of the hypophysis was followed by a vast amount of experimental work on various species of animals (dogs, cats, rabbits, rats, chickens, frogs, toads and amphibian larvae) for the purpose of reproducing this state of skeletal overgrowth through the removal of the pituitary gland by diverse operative procedures. The early attempts to remove the gland by the use of the cautery, curette and acids, were crude, and considerable trauma to the brain occurred, and no doubt many of the resultant effects were not wholly due to removal of the gland, but in large part, perhaps, to a cause quite independent of endocrine underfunction. Furthermore, all the gland substance may not have been removed. Even with considerably more refined surgical technique and post-mortem histological verification of complete hypophysectomy, the question as to whether the pituitary gland is essential to life in mammals remains unsettled. The results of one set of investigators—Horsley and Handelsman, Gemelli, Aschner, Sweet and Allen, Camus and Roussy, Brown and Dandy and Reichert—support the contention that the pituitary gland is not essential to life, while the work of another group—Paulesco, Reford and Cushing, and Crowe, Cushing and Homans, Houssay and

Hug, Bell and Dott—indicates that the gland is essential to life. The question must be left an open one for the present. Even the most recent work of Dandy and Reichert does not settle it. True enough, these surgeons have improved the operative technique, especially by making use of Weed and McKibben's observations that the intravenous injection of concentrated sodium chloride solution withdraws fluid from the brain and causes it to shrink without thereby causing any notable injury. With the brain shrunken the approach to the pituitary is facilitated and the danger of injury to the adjacent structures is minimized. With this procedure Dandy and Reichert have succeeded in performing complete hypophysectomies on dogs and puppies, and their animals have survived for periods considerably longer than those reported by previous investigators—the longest survival period of their adult dogs is 237 days, while in the case of a single puppy it is nine months. Nevertheless the periods are not long enough and their series of animals not sufficiently numerous to settle with certainty so important a question as the essentiality or not of the hypophysis to life, when by life we mean living over the *normal* life-span for a given species. Adult animals respond differently than do puppies to complete removal of the pituitary gland.

Whether the pituitary gland is essential to life or not carries with it so many implications, and, so to speak, so many inseparable issues that it cannot be answered without taking into account these inseparable issues. Earlier in this review reference was made to the presence of hypophyseal functioning tissue in the floor of the third ventricle—an observation first made by Houssay and confirmed by us—and also to the existence of a pharyngeal hypophysis. May it not be that in such animals that survive the surgical removal of the pituitary gland, the above-mentioned accessory hypophyseal tissue is sufficient to supply the minimum physiological needs of the animal and permit it to live? Or, in view of the intimate interrelation of the various internal glands, may not some of the other glands compensate for the pituitary body in hypophysectomized animals? If either or both of these exigencies occur, then the smaller number of animals (adults) which survive hypophysectomy without any ill-effects would form a special group, a small one at that, in which the accessory tissue or the compensatory function of the other glands is sufficient to provide for the animals' needs. In such cases the gland proper could be regarded as unessential to life.

The question as to the essentiality of the gland for life is after all

one of academic importance only, as compared with the more important question: has the gland a functional significance in the animal economy and what is is the exact nature of its function or functions, quite aside from a possible compensatory action of accessory tissue of the same nature?

Investigators, however, are generally agreed that removal of the whole gland or the pars anterior of the hypophysis results in a marked inhibition of growth in young animals due to lack of the growth-promoting hormone. It is in regard to the chain of symptoms, such as cachexia hypophyseopriva, adiposity, genital atrophy, polyuria, etc., following total or partial ablation of the pars anterior, or the whole pituitary body that there is sharp disagreement among investigators. (The posterior lobe can be removed without serious disturbance to the animal organism.)

Crowe, Cushing and Homans, in agreement with Paulesco, find that complete removal of the anterior lobe or the whole hypophysis leads inevitably to death of the animal, with a peculiar and characteristic train of symptoms termed cachexia hypophyseopriva. To ensure that the results obtained were not due to surgical trauma or post-operative complications control animals were subjected to the same operative manipulations except for the single step of removing the gland, and such animals showed no symptoms whatever. When the gland is incompletely removed, no immediate disturbances result. Post-mortem histological examination was made to verify total or partial removal of one or both lobes of the gland.

In addition these authors state that

> separation of the hypophyseal stalk, owing to circulatory disturbances, is comparable either to a partial hypophysectomy or to a total removal with immediate reimplantation of the excised tissue elsewhere in the body. The gland becomes reattached and the pathways for posterior lobe secretion (supposed to traverse the pars nervosa on its way to the infundibular cavity) may become obstructed by the scar, leading to an accumulation of 'hyaline' within the channels of the pars nervosa.
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> Definite constitutional disturbances which we may regard as manifestations of hypopituitarism have been observed after partial (anterior lobe) removals in a number of animals kept under observation for long periods of time. The most striking feature is a state of adiposity accompanied by (or resultant to) a secondary hypoplasia of the organs of generation in adults or by a persistence of sexual infantilism in case the primary hypophyseal deficiency antedates adolescence. Polyuria, glycosuria, alterations in the skin and its appendages (such as oedema and hypotrichosis), the tendency to a subnormal body temperature, and psychic disturbances are more or less frequent accompaniments—all of them symptoms

which occasionally occur with states of adiposity and of sexual infantilism in man, in company with certain pituitary tumors—states, therefore which presumably are due to hypophyseal (anterior lobe) deficiency.

Animals, in states of hypopituitarism, appear to have a lowered resistance to infection, exposure or disease, conditions which are apt to precipitate the onset of cachexia hypophyseopriva.

The experiments of Blair Bell substantiate, in the main, the findings of Crowe, Cushing and Homans but he differs from them in regard to the causation of the syndrome of dystrophia adiposo-genitalis, which he could not produce by the partial removal of the anterior lobe, but which, he found, followed in all cases of compression or separation of the infundibular stalk. Such a procedure interferes with the blood supply of the whole gland, and would also interfere with the passage of the posterior lobe hormone into the cerebro-spinal fluid (assuming such to be its mode of exit). It is to this interference of the blood supply which in turn prevents the escape of the hormones from their seat of origin into the body (in the case of the posterior lobe secretion either by way of the blood directly or indirectly through the cerebro-spinal fluid) that Blair Bell lays the cause of the dystrophia adiposo-genitalis. He thinks it is largely due to an insufficiency of the anterior lobe, the cells of which become shrunken, atrophic and discrete—a similar histological picture is seen in human beings affected with this disease, or in hibernating animals. Blair Bell is of the opinion that the posterior lobe secretion does not go by way of the cerebro-spinal fluid, but leaves by the blood stream from the pars intermedia where it is secreted, hence removal of the posterior lobe does not necessarily interfere with the entrance of the "posterior lobe" hormone into the circulation. But compression or separation of the stalk interrupts the circulation of the whole gland and prevents the escape of both the anterior and posterior lobe hormones. May not the fact, too, that there exists tissue in the floor of the third ventricle (Houssay, Abel and Geiling) capable of elaborating the posterior lobe hormone be of significance in explaining the absence of symptoms following the removal of the posterior lobe? This hypothalamic tissue may form enough of the posterior lobe secretion to satisfy the minimum physiological needs and thus prevent the onset of symptoms referable to deficiency of the post-pituitary hormone. If this syndrome is due mainly to an interference with the escape of both the anterior and the posterior lobe hormones into the body, then one may conceive that Crowe, Cushing and Homans, while removing a part of the anterior lobe of the hypoph-

ysis, interfered in some way with the escape of the posterior lobe hormone into the circulation, or perhaps that removal of the anterior lobe in some way influenced the secretion or action of the posterior lobe hormone (Smith and others thinks removal of the anterior lobe brings about a deficiency in the posterior lobe hormone). Furthermore, the fact that only certain of the symptoms of dystrophia adiposogenitalis, such as low blood pressure and increased carbohydrate metabolism, are alleviated by injection of the posterior lobe hormone, while the preliminary work of Smith, Walker and Graeser, to be discussed below, indicates the correction of genital atrophy by injecting anterior lobe extracts into hypophysectomized rats, points to the conclusion that the syndrome is referable to a deficiency of both the anterior and the posterior lobe hormone. It is, of course, risky to make such deductions as the foregoing especially since the experimental data were obtained with different species of animals; they are, however, only made in an attempt to correlate the findings of different workers.

Dott's work confirms the findings of Crowe, Cushing and Homans and of Blair Bell and in addition he finds that mechanical irritation of the pars intermedia through insertion of a platinum plate brings about a very marked polyuria. He suggests that hypophyseal polyuria experimentally produced is dependent on an *irritative stimulation* of the pars intermedia. May not an irritation in this neighborhood be instrumental in reducing the output of the pars intermedia?

Some preliminary experiments by Smith, Walker and Graeser furnish strong evidence in support of the view that the dystrophia adiposogenitalis syndrome is connected with pituitary insufficiency. These investigators report the production of the above named disease entity in rats through the destruction of the pituitary gland by injecting into it a 10 per cent solution of chromic acid by means of an exceedingly fine needle. The gland is entirely destroyed, and destruction of the adjoining tissue is prevented by the capsule of the pituitary. (These investigators no doubt have verified this statement by histological sections.) Seven rats subjected to this treatment developed the well-known Fröhlich's syndrome and several others showed a disturbance in the reproductive system.

One rat, a female, operated upon February 17, failed to show the usual sex cycles, although displaying no adiposity. On May 22, one ovary and a part of the corresponding horn of the uterus were removed for diagnosis. These structures were found to be much smaller than normal. The ovary contained no corpora and no follicles approaching maturity. There was considerable interstitial

tissue. After allowing a few days for recovery from the operation a daily injection of anterior hypophyseal substance by the method of Evans and Long was begun on May 28. Ovulation revealed by the vaginal smears took place on June 10 and June 14, the usual four day cycle thus being displayed. Autopsy on June 17 revealed a normal sized ovary with many corpora and large follicles. The uterus had become normal in size. No pituitary could be distinguished with the binocular. A study of serial sections revealed the presence of apparently normal neural and intermediate lobe but no typical anterior lobe cells.

It thus seems clear that in this case we had caused the ovaries to become structurally atrophic and functionally inactive by injuring the pituitary body, and that a repair was effected by the administration of anterior lobe substance, a result which it would seem removes in this case the possibility of a brain lesion being the causative factor except in so far as such a lesion might have directly contributed to the impairment of the hypophysis.

These investigators are cautious, and if further confirmation is forthcoming the results no doubt will be very valuable in helping to establish the hormonal conception of some of the disturbances ascribed to pituitary insufficiency. The experimental procedures are especially useful in that they show the way to overcoming pituitary insufficiency by substitution organotherapy (a method of experimentation which as yet has not been very successful in pituitary disturbances). Evans in his Harvey lecture states that Smith and collaborators have verified the above results on twenty animals.

A further consideration, and one which is especially applicable to clinical conditions, may be touched upon. In view of the fact that there is a hormone in the anterior lobe and another one with a different function in the posterior lobe or pars intermedia, may it not be that some of the pathological conditions associated with the gland, whether produced experimentally or observed clinically, are the result of varying degrees of deficiency or over-production of one or both of the hormones? If such be the case we may have all grades of either a hyper- or a hypofunction of one or both of the lobes and even a mixed disturbance, thus rendering a sharp allocation of the source of disturbance very difficult or impossible. For this reason the term dyspituitarism (perverted function of the hypophysis) used by Cushing is very useful. This point seems extremely important since new knowledge is almost daily coming to light to show the very remarkable interdependence of the various ductless glands, and how disturbance in the function of one gland may in turn influence that of another.

Another group of workers—Camus and Roussy, Aschner, Houssay, Bailey and Bremer, Dandy and Reichert—maintain that the symptoms described in the preceding paragraphs can all be produced by injury

to the hypothalamus or to the brain, and without in any way damaging the pituitary gland. Bailey and Bremer, employing the surgical route of approach first used by Paulesco and later perfected by Cushing and his associates (Reford, Crowe and Homans), made in dogs hypothalamic punctures of almost microscopic dimensions, and without in any way injuring the hypophysis, produced in these animals symptoms of transitory polyuria, or of permanent diabetes insipidus, of dystrophia adiposo-genitalis, or of rapid genital atrophy with so-called "cachexia hypophyseopriva." The symptoms observed depended in their intensity strictly on the situation of the lesion in the tuber cinereum and on its dimensions. An extensive lesion of the tuber cinereum is incompatible with life; the animals die either quickly or after a period of apathy in coma and convulsions. Glycosuria was an inconstant result of the lesion in the tuber cinereum. Deep lesions made outside of the region in which these important nerve centers (injury of which is ascribed as the cause for the symptoms mentioned above) are located never caused a polyuria. The diabetes insipidus experimentally produced does not depend on a disturbance of a supposed nervous or vascular regulation of the kidneys, because the symptoms persist even after denervation of the kidneys, and may be induced in dogs with denervated kidneys by puncturing the tuber cinereum.

These investigators do not offer a satisfactory explanation for the fact that post-pituitary extracts are efficacious in alleviating these symptoms. Attempts to reconcile these seemingly divergent results, or to point the way for a compromise of controversial points are only of avail when they are made with accurate experimental data. To illustrate, should the syndromes as typified by the work of Crowe, Cushing and Homans, be due to injury of the hypothalamus, then their findings would be in agreement with those of Camus and Roussy and Bailey and Bremer and others. On the other hand, if the pituitary gland was injured during the experimental procedures of puncturing the hypothalamus, then the results of Camus and Roussy and others may be interpreted in terms of pituitary gland injury. Assuming, however, that both sets of experimenters have interpreted their findings correctly (and such an assumption is an entirely fair one) we have two apparently contradictory sets of data. How can they be reconciled? Of the possibilities that suggest themselves the following is one: In order to prevent adiposity, glycosuria, diabetes insipidus, etc., both the pituitary gland and the hypothalamus must be intact; injury to either will suffice to bring on the conditions above mentioned

which may be regarded as disturbances in the regulatory functions of the body.

Abel in his Harvey Lecture makes the following comment on the work of Camus and Roussy and Bailey and Bremer and others:

> Before accepting such an explanation, it would have to be shown that puncture of the hypothalamus in the immediate neighborhood of the upper attachment of the hypophyseal stalk in no wise interferes with either the production of the hormone in the pars intermedia or pars posterior, or with its transference to the cerebro-spinal fluid or to the blood stream or both. It is a curious fact that in frogs a puncture of the brain, trifling in itself and followed by no observable symptoms, can nevertheless be shown to have caused certain profound alterations in the response of the frog's brain and spinal cord to a whole series of sulphonated convulsant dyes. In past years my collaborators (Syz and others) and I have found that various trifling experimental lesions of the frog's brain cause this organ to take up such dyes in great abundance, and convulsions are produced in these animals by doses very much smaller than would be required to produce the same symptoms in normal animals. Furthermore, such frogs differ from normal ones in that their brain and spinal cord take and retain a great deal of the injected dyes. It is therefore within the range of possibility that puncture of the hypothalamus is not without an effect on the functional integrity of the pars posterior and pars intermedia.

Schafer, too, in a recent lecture finds it difficult to accept the view of Camus and Roussy, Bailey and Bremer and others and thinks that punctures, even minute ones, in the floor of the third ventricle may interfere with the normal functions of the gland; either 1, through the vascular supply; 2, through hitherto undiscovered nervous connections; 3, through interference of the passage of the products of secretion of the pars intermedia into the ventricles.

In the tadpole and the salamander the effects of early hypophysectomy seem clear-cut and conclusive as is shown by the work of Adler, Smith and Smith, Allen, Houssay, and Huxley and Hogben. The term hypophysectomy in the case of the tadpole operations is restricted to the removal of the epithelial or buccal anlage only which is easily accessible from the exterior and not united to the brain, from the ventral surface of which it can be cleanly dislodged at the time when these animals are operated upon. Removal of the anterior lobe only in tadpoles at an early stage causes an undersized neural lobe with deficient secretion. (Holt is inclined to throw doubt on the view of Mihalkovics (1875), Herring (1908), Smith (1920), that the neural lobe develops well only when it is in contact with the glandular lobe since he observed a pig embryo of 40 mm. in which the pars buccalis

was totally lacking, while the neural part, the thyroid, the suprarenals and the gonads were well developed.)

Removal of the buccal anlage or anterior lobe of the hypophysis in early embryos is not incompatible with life and causes the following changes; 1, a slowed rate of growth; 2, an underdevelopment of other endocrine glands, the interrenal bodies, the parathyroids and thyroid, with a consequent failure of the larvae to metamorphose; 3, an abnormal condition of the pigment-bearing cells giving rise to albinism; and 4, a large and persistent fat organ which probably indicates a disturbed metabolism. Greenwood finds that removal of the pituitary gland in the larvae of the Mexican salamander when the thyroid is fully developed does not lead to very decided alteration in the growth rate, as compared with that of normal specimens.

The results with substitution organotherapy have also been singularly successful and are summarized by Smith and Smith as follows:

> The intraperitoneal injection of different portions of the bovine hypophysis in the hypophysectomized tadpole gives the following results:
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> 1. Pars anterior brings to normality the four deficiencies resulting from hypophysectomy in that it accelerates the growth rate; induces a normal depth of pigmentation; repairs the atrophic thyroid and interrenal bodies, induces metamorphosis and reduces to normal dimensions the fat organ. The normal and thyroidless tadpoles when injected with anterior hypophysis show an acceleration in their growth rate, attaining an unusually large size, and display an increased depth of pigmentation.
> 2. Pars intermedia corrects the pigment upset and reduces the size of the fat organ.
> 3. Injections of posterior lobe extracts into "pituitaryless" tadpoles corrects the adiposity and albinism of hypophysectomized tadpoles.
> 4. Extracts made from the solid colloidal deposits which are found between the anterior and posterior lobes (Fenger) do not contain any physiologically active material as determined by changes which it may cause when injected into hypophysectomized tadpoles.

Transplanation Experiments. Halsted's dictum that an existing "physiological deficit" is one of the essentials for organo-transplantation has only in part been verified by the experiments of Crowe, Cushing and Homans who have been successful in prolonging the life of hypophysectomized dogs by immediately reimplanting the excised gland into the cerebral cortex. They have also tided their animals over periods of threatened "cachexia hypophyseopriva" (an invariable result of anterior lobe or total removal of the gland) by leaving in situ fragments of anterior lobe tissue or injecting anterior lobe extracts.

Dott finds a large "safety excess" of anterior lobe tissue—two-thirds of the gland can be removed before symptoms of deficit appear. Other investigators (Clarimont and Ehrlich, Schafer and Exner) obtained negative results with gland transplantations, or only very transient effects due probably to the absorption of the active principle from the implanted gland.

Allen, working with tadpoles, succeeded in transplanting the anterior lobe of adult frogs into normal, into hypophysectomized and into thyroidless tadpoles by placing the transplant in a pocket under the skin above the right eye, and thereby produced a marked acceleration of growth so that the treated tadpoles were conspicuously larger than those into which the other parts of the hypophysis had been transplanted. They were larger than normal controls into which muscle tissue had been similarly engrafted. The anterior lobe caused a marked acceleration in the development of the hind legs. Swingle, working independently of Allen, obtained results which agree essentially with those mentioned above.

Before proceeding to a discussion of the physiological rôle of the posterior lobe of the hypophysis we may mention that investigators are agreed that extracts of the anterior lobe when injected into anesthetized mammals cause a fall in blood pressure.

THE POSTERIOR LOBE OF THE HYPOPHYSIS. In the foregoing pages several points connected with the posterior lobe were touched upon and they may be briefly summarized here. The origin of the "posterior lobe" hormone does not appear to be quite settled; a generally accepted opinion is that the hormone comes from the pars intermedia which contains secreting cells and which covers and in places dips into the posterior lobe. The route by which this hormone leaves is still an open question; that is, whether it goes directly into the blood stream or goes through the third ventricle into the cerebro-spinal fluid. This issue will be settled satisfactorily only when it can be demonstrated that the substance in the cerebro-spinal fluid has the same chemical and multiple physiological properties as those possessed by the posterior lobe hormone; and that the hormone is present in the cerebro-spinal fluid in larger concentration than it is in the blood stream. Such proof will, no doubt, be forthcoming when a very delicate chemical method for the quantitative determination of the principle under discussion will have been worked out.

Investigators are agreed that the posterior lobe may be removed without any apparent ill effects to the animal. This fact may be

explicable on several grounds: 1, removal of the posterior lobe (it being largely neuroglia tissue) may not interfere with the pars intermedia from which the hormone is secreted; and the hormone may find an exit into the systemic circulation, either by way of the blood stream or through the cerebro-spinal fluid; 2, the hypophyseal functioning tissue in the hypothalamus may secrete enough of the hormone to enable it to play its normal rôle in the body (the amount may be minimal, to be sure, yet sufficient to satisfy the minimum needs); 3, the functions of this hormone being largely regulatory may be assumed or compensated for by the secretions from other glands; or 4, the body may be able to adjust itself, so as to function apparently normally without this regulatory hormone.

Several investigators report loss of weight following the feeding or injection of whole gland extracts, which they ascribe to the presence of the posterior lobe hormone.

It is an accepted fact that the injection of extracts made from the posterior lobe of the hypophysis calls forth a very marked physiological response which manifests itself on the cardio-vascular, renal, smooth muscle, and certain glandular structures in the body, producing:

1. A sharp and sustained rise of the arterial blood pressure (except in the duck). Repeated injections have not only little or no pressor action, but may produce an inversion of the blood pressure—a depressor response.
2. Diuretic-antidiuretic action.
3. Stimulation of the uterus, intestines and bladder.
4. Secretory accelerator effect on the mammary gland, and an inhibitory action on the salivary gland.

In amphibians post-pituitary extracts also have a very pronounced action on the pigmentary system—a melanophore-expanding action. Certain minor effects will be mentioned later.

It may be appropriate as a preliminary to the discussion of the various physiological actions of infundibular extracts to emphasize here that the autacoid in such extracts is an extremely delicate substance and very easily injured by apparently mild chemical reagents. Owing to this fact it is certain that some investigators used partially injured material. Furthermore, aside from the active principle, post-pituitary extracts contain varying amounts of depressor bodies (Schafer and Vincent and Abel and Nagayama) which may have a

modifying action. The physiological effects of infundibular extracts depend, too, on the size of the dose. These facts and others to be mentioned are of value in enabling one to appreciate the number of seemingly divergent results obtained by numerous workers with extracts prepared in different ways (some undoubtedly injurious to the active principle) and used in very different concentrations and with a variety of animal species.

Chemical Principles in the Posterior Lobe of the Pituitary Body. In marked contrast to the scant amount of work done on the chemistry of the anterior lobe stand the chemical studies of numerous workers to determine the number and nature of the active constituents of the posterior lobe of the hypophysis. At present investigators are sharply divided in their opinions as to the number of principles present, and as the differences, at the moment, appear irreconcilable all that can be done here is to present the views of the various workers with such comments as are pertinent, and the rest must be left for future classification and settlement.

The work of the early investigators, which at present is of historical interest only, will be found discussed by Houssay in his monograph as also in a paper by Fühner who maintains that there are four crystalline principles separated from the posterior lobe extracts by the chemists of the Farbenfabriken Hoechst a. Main. These assumed crystalline principles are at present generally believed to have been sulphates of certain tissue constituents on which were adsorbed varying amounts of what is now known to be a principle of extraordinary potency. Dale and Dudley claim that by the use of butyl alcohol the oxytocic and pressor principles are separable. Abel and his co-workers are unable to accept the point of view of Dale and Dudley. They were unable to effect any appreciable separation of oxytocic and pressor action of their pituitary tartrate by using dry n-butyl alcohol as a differential solvent. On the other hand watery solutions of n-butyl alcohol dissolve the tartrate completely. Hogben and Winton state that the melanophore and uterine activity is probably contained in one principle; the melanophore action is rapidly destroyed by tryptic, but not by peptic digestion, and is inactivated by boiling with 0.5 per cent HCl at about the same rate as the oxytocic principle, but much more slowly than the pressor principle. On the other hand Dreyer and Clark claim that the melanophore stimulant can be partially separated from the oxytocic principle by ultra-filtration, while Fenn

claims to effect the same separation by the use of butyl alcohol. Dreyer and Clark and Fenn in agreement with Hogben and co-workers also conclude that the pressor and oxytocic principles are distinct.

Heymans compared the oxytocic and pressor actions of a number of commercial samples of pituitary extract, and could find no parallelism between the two actions. This would indicate that there are two separate bodies. It must, however, be mentioned that all his samples assayed much below the strength indicated by the manufacturers. Heymans employed his method of perfusing the isolated rabbit's head for testing the vaso-constrictor action.

The investigators here cited who stand for a multiplicity of posterior lobe principles, it should be noted, base their contention less on extensive methods of purification, than on comparative physiological tests made with very crude extracts. As will be shown later, other investigators, on the basis of similar physiological experiments also made with unpurified extracts, have come to the support of the contention of Abel and his associates that there is only one hormone in the posterior lobe.

Herring asserts that the pressor and oxytocic constituents of the pituitary gland are differently distributed in glands of different species or in the different regions of the gland in the same species. Hogben and de Beer confirm these findings. Certain criticisms may be made in regard to some of the work mentioned above.

Abel and his co-workers have made from the posterior lobe of the hypophysis a preparation in the form of a tartrate which far exceeds in potency any of the fractions obtained by the above-named investigators. While they do not as yet claim entire chemical purity and individuality for their pituitary tartrate, they maintain that this salt contains only one specific principle which possesses practically all the diverse physiological actions of a well-prepared aqueous extract. This principle is extraordinarily potent as a smooth muscle stimulant, being 1000 to 1250 times more powerful than histamine phosphate on the isolated virgin guinea pig's uterus. In addition to its oxytocic action this pituitary tartrate causes 1, prolonged rise in the arterial blood pressure with constriction of the peripheral arterioles and capillaries; 2, a brief diuresis in the green-fed rabbit; 3, an antidiuretic action in diabetes insipidus, and 4, no constriction of the bronchi of the pithed dog (in large doses there is evidence of dilatation); and 5, affects the respiration of rabbits, as also the breathing and the heart of unanesthetized dogs, in a characteristic manner. These manifold

actions, namely, oxytocic, pressor, diuretic-antidiuretic, respiratory and cardiac, are all obtained in high dilution with the purified tartrate.

Further evidence in support of the unitarian conception of the hormone in the posterior lobe of the hypophysis is seen in the following facts:

(a) All of the physiological properties of our active substance increase in intensity and in the same ratio *pari passu* with the methods of purification, from the initial precipitation of the fresh posterior lobe substance to the final precipitation of the tartrate. It is unlikely that all of these physiological properties would retain their relative position to each other throughout a long series of varied chemical processes were they not characteristic of one and the same principle. (b) Exposure of the tartrate to normal sodium hydroxide for one hour at room temperature *completely destroys all* of its physiological action. It is extremely unlikely that so comparatively mild a treatment as this should simultaneously destroy three or four separate principles. Guggenheim in 1914 was the first to show that alkali destroys both the pressor and the oxytocic actions of a pituitary extract and held that this effect alone is sufficient to prove that both physiological actions are properties of one and the same substance. Since 1914 it has been shown by a number of investigators that every known physiological action of the posterior lobe is affected in a similar manner by exposure to alkali. (c) Boiling a solution of the tartrate with 1 per cent hydrochloric acid under a reflux condenser for one-half hour is also destructive of all of these physiological properties with the exception of the oxytocic action, a certain proportion of which appears to withstand this short treatment with acid (Abel).

A number of investigators by entirely different procedures than those used by Abel and his collaborators are coming to the conclusion that the manifold physiological properties of the infundibular hormone are all inherent in one principle.

Thus Leyko and Sikorski studied the blood pressure-raising power of post-pituitary extracts on dogs at intervals of two days, the operation being performed under local anesthesia. The action of the extracts upon the urinary secretion of dogs with a permanent bladder fistula was then studied and it was found, as in their previous experiments (1921), that the extracts promptly check the diuresis induced by the copious administration of water to the animals. The pressor and antidiuretic action of the extracts were then compared with their oxytocic action on the guinea pig's uterus and it was found that "these three actions go together." To cite a passage from the concluding section of their paper: "An extract giving a strong pressor effect acts with about equal strength upon the diuresis and vice versa. This seems to speak in favor of Abel's view that the extract contains only one main active principle acting in all three directions, and against the presence of a number of different active bodies in the gland each acting in only one way." Smith and McClosky studied the diffusion rate of the several physiologically

active constituents of the infundibulum through a series of collodion membranes of graded permeability and conclude that the identical diffusion rate of the oxytocic, pressor and renal activities present in infundibular extracts argues in favor of their chemical identity, and confirms the view held by Abel and his collaborators on this matter (Abel).

These authors also studied the effect of keeping a post-pituitary extract for various periods of time at a temperature of 60° on the pressor and oxytocic properties. One extract which was kept in the incubator for 1½ months at this temperature lost 80 per cent of its oxytocic activity, and, as nearly as could be determined, the same loss was found in its pressor activity. Another extract kept under the same conditions for 3 months retained only a trace of its oxytocic property, namely 6 per cent, and likewise only an undeterminable trace of its pressor activity. It is certainly most improbable that two separate constituents should show in equal degree, such marked sensitivity to so mild a treatment. Much the more likely thing is that the oxytocic and pressor effects are characteristic of one and the same body. Smith and McClosky also find that in their acetone-extracted powder the diuretic, oxytocic and pressor principles are present in the same amounts as in fresh gland extracts. In this connection reference may be made to the work of Coope and Chamberlain who showed that extracts of the posterior lobe of the pituitary body when injected into rabbits and cats produce a well-marked increase in the amount of fatty acid in the liver. This property, as also the oxytocic and pressor activities, are destroyed when pituitary extracts are heated for 6 hours on a waterbath with an equal volume of 1 per cent hydrochloric acid.

Abel and Nagayama some years ago showed that heating freshly prepared infundibular extracts on the bath or boiling them at the reflux condenser with 0.5 per cent hydrochloric acid for a half-hour completely abolishes the pressor activity and 80 per cent of the oxytocic property. Dale and Dudley later confirmed these findings, but noted that after 6 hours' boiling with 0.5 per cent acid practically all the oxytocic action is destroyed.

In addition to the active principle there are depressor bodies present in the posterior lobe of the hypophysis (Schafer and Vincent). Abel and Nagayama have designated two of these blood pressure-lowering substances "B" and "C", from which they separated the active principle "A" which is the purified pituitary tartrate. The "C" substance is

histamine and was isolated by Abel and Kubota in the form of a crystalline picrate.

Whether the melanophore-expanding property belongs to the purified pituitary tartrate has not as yet been established by Abel and his co-workers. Preliminary experiments on hypophysectomized tadpoles and on the frog's foot were made only with relatively impure preparations of their tartrate (160 × β-I) and whether the action on the melanophores was due to the pressor-oxytocic-diuretic principle or to an admixed impurity will have to be determined by using the most highly purified salt.

Action of Enzymes on Post-Pituitary Extracts. It is generally conceded that pituitary extracts do not exercise their optimum physiological action when administered by mouth, although Rees and Olmstead report some success in the treatment of a case of diabetes insipidus where the infundibular extract was given per os, while Morris and Weiss were successful in treating a case of dyspituitarism by administering such extracts by mouth. Similarly Donaldson found pituitrin by mouth of value in cases of uterine hemorrhage.

The majority of investigators (Schafer and Herring excepted) find that pituitary extracts are inactivated by tryptic but not by peptic digestion (Dale, Hamil, Donaldson, Dudley and Hogben), although in their recent studies Rees and Whitehead, who review the literature, conclude, on the basis of their experiments, that neither the gastric nor the intestinal enzymes destroy the pressor and oxytocic actions of infundibular extracts.

Cardio-Vascular Action. The first of the physiological properties of extracts of the pituitary body to be discovered was the action on the arterial blood pressure. Oliver and Schafer in 1895 found that extracts of the pituitary gland when injected intravenously into anesthetized dogs cause a sharp and prolonged rise of the general arterial blood pressure. Howell later showed that this pressor substance is confined to the posterior lobe of the hypophysis. He also noted a slowing of the heart rate and that a second injection given some time after the first one had a very much lessened action. Von Cyon, too, observed that the rise of blood pressure is accompanied by slowing of the pulse. He thought that these extracts when injected stimulated the pituitary body. Accordingly he applied electrical stimulation to the gland and obtained similar effects. After removal of the gland, he claims, the effect can no longer be produced by injection of gland

extracts. Schafer and Vincent found that the administration of a second or third dose of pituitary extract always produces a fall, and not a rise of pressure. This point will be discussed in greater detail below.

That the rise of blood pressure is due to a peripheral vaso-constrictor action is now generally accepted, the action being directly on the smooth muscle coat of the vessel (Dale).

Tests with isolated strips of arteries show that a marked constriction of the coronary, pulmonary carotid, mesenteric, femoral, and peripheral end of the renal artery occurs under the influence of pituitary extracts (Pal, De Bonis and Susanna, Dale, Campbell, and Morawitz and Zahn). It is interesting to note here that Brodie and Dixon observed that the muscular coats of the smaller arteries of the lung are not under the control of the sympathetic nervous system. Epinephrin does not cause these vessels to constrict but pituitary extracts do. The coronary arteries, too, are thought not to be under sympathetic control (Schafer, Elliot). Cow finds a dilatation of the gastric, hepatic and splenic arteries; this dilatation decreases progressively from the viscus to the origin of the vessel from the coeliac axis, where a constriction may be obtained in the neighborhood of the origin of these vessels. He finds the renal artery dilated, and that the dilatation increases progressively in the same artery from its origin from the aorta to its branches within the kidney. In a recent study Sumbal observed that in the tortoise the coronary vessels are dilated by pituitary extracts either when this is applied locally or added to the perfusion fluid. This observation is significant since in the species of animal used by him, pituitary extract causes a fall in blood pressure, while in mammals the coronary vessels are constricted and the blood pressure is raised under the influence of infundibular extracts.

A marked reduction in the pulse volume follows injection of infundibular extracts. Hoskins and Means demonstrated this by placing the central stump of a ligated carotid artery in a small plethysmograph and registering the excursions made by the pulsations within this portion of the artery. Kolls and Geiling further investigated the reduction in pulse volume by using a modified Gasell maximum-minimum valve to determine the maximum and minimum pressures in etherized dogs, and found an extreme reduction in pulse pressure during the time the mean pressure is elevated. In this connection the changes in systolic and diastolic blood pressures were determined in trained dogs without anesthesia by using Koll's sphygmomanometer, and it was

found that the reduction in pulse pressure is relatively greater in the case of the unanesthetized animals. (It may not be amiss here to emphasize again the great desirability of conducting experiments wherever possible on the unanesthetized animal, especially as recent studies of various experimenters show the very profound changes in the metabolism produced by anesthetics such as ether and chloroform. In studying drugs with anesthetized animals one must remember that there is obtained the combined action of anesthetic plus drug—the net result at times being very different from that obtained with the drug injected into an unanesthetized animal (Geiling and Kolls). It is fair to say that much of the existing physiological and pharmacological knowledge will be modified or even perhaps reversed as more of our known drugs are studied on unanesthetized animals. Furthermore by adopting some of the newer methods of experimentation much information formerly obtained after a mutilating operation and prolonged anesthesia, can now be secured by using simple non-injurious procedures on trained subjects, especially dogs and rabbits. There are of course, objections to the use of the decerebrate cat, too well known to be more than mentioned here.)

Carrier found that the subcutaneous injection of pituitrin in dilutions of 1:100 causes constriction of the human capillaries. Krogh and his associates have shown in their frog experiments that capillary tone is controlled by an internal secretion from the hypophysis (posterior lobe secretion). Removal of the pituitary body from the frog is followed by a marked dilatation of the capillaries in the skin and the web of the foot. Several weeks after the hypophysectomy some degree of constriction of the vessels is restored but the circulation through the capillaries is irregular. Pituitary extracts administered to such animals cause the capillaries to contract.

Abel, Rouiller and Geiling call attention to the marked paling of the skin and visible mucus membranes in white unanesthetized dogs following the injection of their highly potent pituitary tartrate. Dispersed throughout this pale surface are distended veins which stand out prominently. Kolls and Geiling using Lombard's method obtained photomicrographs of the small vessels on the inner surface of such an animal's ear which showed in all cases a very marked constriction of the vessels, thus demonstrating that pituitary liquid contains a very potent capillary-constrictor principle. Sachs in a recent contribution noted paling of the skin as a constant feature after the intravenous injection of small doses of pituitary extract into human

subjects. He concluded that the paling is due to constriction of the capillaries, collecting venules and subpapillary plexus. Capps and Mathews observed an increase in the venous pressure following administration of pituitary extracts. We have observed in unanesthetized dogs previously injected with heparin (to prevent coagulation of the blood) that pituitary liquid, as also the potent pituitary tartrate, produce a considerable rise in the venous blood pressure. This elevation of the venous pressure coincides in time with the paling of the skin and distention of the veins above referred to.

Not all species of animals show a pressor response to the first injection of infundibular extracts as was demonstrated by Paton and Watson who observed a fall in blood pressure in the decapitated duck after the first injection of commercial pituitary extract. This observation has been confirmed by Hogben and Schlapp who in addition have extended their studies to the blood pressure response to pituitary extracts throughout the vertebrate series. They found that pituitary extracts which have no depressor action in the mammal consistently evoke depression in birds and reptiles (duck, fowl and tortoise). In frogs large doses produce a slow steady rise of blood pressure, comparable to that produced by epinephrin. Sumbal has recently confirmed Hogben and Schlapp's finding that the pituitary extracts produce a fall of blood pressure in the tortoise. Schafer and Vincent found that the second or third dose always produced a fall of pressure. Later Vincent and his collaborators demonstrated that an alcohol-soluble depressor substance is present not only in pituitary extracts but is found in extracts of all organs and tissues. Hogben and Schlapp have recently taken up the question of the cause of the fall in blood pressure following the second or subsequent injections of pituitary extracts. These investigators confirm the finding of Schafer and Vincent and further claim that pituitary extracts prepared from desiccated glands freed of depressor substance no longer invoke inversion of the blood pressure upon repeated injection. They maintain that the inversion phenomenon obtained by Abel and his associates with their highly purified pituitary tartrate is not an intrinsic property of their body, or the hormone, but is due to the presence of the depressor substance which has not been completely removed. Hogben and Schlapp's contention is quite unwarranted. Abel and his associates are well aware of the presence in pituitary extracts of alcohol-soluble depressor substances which, however, are removed very early in the process of

preparation of their pituitary tartrate. The following statements taken from Abel's Harvey Lecture are pertinent at this juncture.

> This salt (the pituitary tartrate) is quite free from all preformed depressor substances which are usually mixed with it in the ordinary commercial extracts. As has been stated in the description of the chemical processes, these accompanying depressant substances are completely separated during the early stage of those processes. As a consequence of the absence of depressant substances, the arterial tracing, following an intravenous injection of the tartrate, generally mounts straight upward and does not show that pronounced downward dip which is so characteristic of the tracings obtained with pituitary extracts which have suffered injury in the course of preparation. Even with tartrates of the lower oxytocic titer of say 40 to 75 times β-I phosphate we only rarely find a small downward dip preceding the steep rise in pressure, and it is probable that this finds its cause in too rapid an injection of the solution. . . . . This inversion effect is not invariably obtained; the same solution may give the effect in one animal and not in the next. It appears to depend upon a number of indeterminate factors such as the depth of anesthesia, the relative size of the first and subsequent doses, the number of and time interval between successive injections, and possibly the age of the animal. I cannot believe that this inversion effect can be explained by assuming that the tartrate is a mixture of two substances, one possessing a pressor and the other a depressor action, and that the action of the latter becomes manifest only when the vascular apparatus can no longer respond to the former. Against this view the following facts may be adduced. The purified pressor substance, as I have frequently pointed out, is easily injured by various chemical procedures so that its characteristic action is lost and a depressor effect appears in its place. Now in cases where the injury is only partial, that is to say, long before all of the pressor substance has been destroyed, a preliminary depressor effect is obtained—an effect which is apparently proportional to the amount of the pure pressor substance which has been inverted. So also if a very small amount only of histamine (something above an inoperative minimum) be added to our pure tartrate, its presence is alway detectable by the fact that the arterial tracing shows, when this mixture is injected, a definite downward dip before the rise.

Furthermore, unpublished experiments of the writer show clearly that even after a defatted pituitary powder has been extracted 48 hours in a Sohxlet with absolute alcohol, as described by Hogben and Schlapp, it will still produce, when injected into etherized cats, either with intact or with cut vagi, and in the same doses as used by Hogben and Schlapp, a lessened response with the second injection, and later injections will effect the inversion. Undoubtedly the presence of depressor substances will facilitate the fall in blood pressure on repeated injection of pituitary extracts but Hogben and Schlapp are in error when they assert that the fall in pressure is due only to the de-

pressor substance and is not an intrinsic property of the infundibular hormone as is maintained by Abel and his associates.

The reversal of the initial effect on the blood pressure obtained upon repeated subsequent injections of pituitary extracts does not stand as an isolated instance in pharmacology. And to cite only one here, we may refer to the work of Cloetta and Waser who found a striking difference in blood pressure response between the first and subsequent injections of *ac*-tetrahydro-β-naphthyl-amine. The first injection of this base elicits a more or less powerful pressor response while the second injection produces a distinct and often considerable, although transitory fall without any subsequent rise of blood pressure. Furthermore the pressor action of a drug may be completely reversed by the previous injection of another drug. We may cite the case of the fall in blood pressure caused by the injection of epinephrin where a previous injection of ergot (Dale) or ergotamine (Rothlin) has been made.

The initial injection of an infundibular extract, or the pituitary tartrate—very potent drugs to be sure—causes a very profound physiological alteration in the experimental animal—the blood vessels are constricted, the cardiac rate and output changed, the nutrition of the heart is probably interfered with, the smooth muscles throughout the body are affected, the ventilation of the animal may be altered. Now the rapid administration of a large dose of this powerful hormone into such an altered animal (the conditions under which we obtain the "inversion effect") cannot be expected to give the same response as the initial dose, since the conditions are different—we have, as it were, a changed subject or a prepared animal. Simply conceived, the "inversion effect" results where the initial injection of a potent pressor drug (the post-pituitary principle in this case) so alters the physiological state of the experimental animal that subsequent larger doses of the same powerful principle administered shortly after the first one, and rapidly, no longer have a pressor action, but elicit a depressor response.

Cardiac Changes. The work of Wiggers, McCord and Dale and others indicates that there is a certain amount of cardiac dilatation following the injection of pituitary extracts. Dilatation of the heart caused by pituitary liquid injected intravenously into unanesthetized dogs has been noted by Kolls and Geiling in fluoroscopic examinations and in teleoroentgenograms. The significance of these changes is at present not entirely clear. Certain very marked changes in the circulation are also occurring during the time that the heart is undergoing these alterations. The pulse is slowed to about 30 or less beats per minute,

the diastolic pressure is elevated, and the venous pressure may be elevated. Sachs found no measurable change in the dimensions of the cardiac outline in three human subjects who received infundin intravenously. Out of fourteen patients ten showed some slowing of the cardiac rate.

Tigerstedt and Airila some years ago found, by means of Tigerstedt's stromuhr inserted into the aorta of an anesthetized rabbit, a diminished cardiac output (minute volume) as a result of the intravenous administration of pituitary preparations. Such an action of pituitary extracts has been inferred by others from experiments with the cardiograph and the cardiometer.

From the results of a number of studies made upon the action of pituitary extracts on the mammalian heart certain facts seem to be well established in relation to the mechanism whereby the slowing of the cardiac rate, following injection of infundibular extracts, is brought about. The work of previous investigators on this topic is reviewed in a recent contribution by Resnik and Geiling who studied the action of pituitary liquid (Armour) and of purified pituitary tartrate on the heart of the unanesthetized dog by the use of the string galvanometer. The weight of evidence seems to favor the view that post-pituitary extracts affect the heart not only through stimulation on the cardio-inhibitory center, but also through a direct action on the myocardium (Wiggers). Resnik and Geiling find that the intravenous injection of 1 cc. of pituitary liquid into an unanesthetized dog causes the following cardiac changes: a brief period of acceleration; a profound slowing with prolongation of auriculo-ventricular conduction time. During this phase, electrocardiographic curves show so-called sino-auricular block. After atropin, pituitary extract brings about an early period of acceleration, followed by a marked fall in rate, often associated with slight depression of auriculo-ventricular conduction. Following this there occurs a progresive fall in rate, independent of the influence of vagal tone. From the above observations, they conclude that under the conditions of their experiments, pituitary extract affects the heart by stimulation of the vagi through the cardio-inhibitory center and by a direct action upon the myocardium. There is possibly an indirect action on the sympathetic nerve endings during the secondary rise, through stimulation of the adrenals by asphyxia. They call attention to the similarities between the effect of pituitary extract and anoxemia of the heart.

With the same procedures and using the purified pituitary tartrate

of Abel and his co-workers, Geiling and Resnik find that this salt produces exactly the same changes in the heart of the unanesthetized dog as those observed after the administration of pituitary liquid (Armour).

The property of post-pituitary extracts to raise the blood pressure in mammals has been suggested as an accessory method for the standardization of this drug, which, while not so delicate as the virgin guinea pig's uterus method, may serve as a useful check of the latter method (Dale and Laidlaw, Hamilton and Rowe, Roth, Houssay, Burn and Dale, Smith and McClosky, Hogben, Schlapp and MacDonald, League of Nations Health Organization Memorandum on Standardization of pituitary posterior lobe extracts).

Perfusing the rabbit's ear with cold Ringer's solution has also been suggested as an additional method of assay for the vaso-constrictor effect of pituitary extracts (Von Schlimpert and von Rischbieter). Heymans, too, recommends perfusion of the isolated rabbit's head with warm Ringer's solution as a method of testing the vaso-constrictor activity of post-pituitary preparations.

Smith and McClosky and Hogben and Schlapp and MacDonald have recommended refinements in the technique of the pressor methods used by them for standardizing pituitary extracts which enable them to obtain checks comparable to those secured with the uterine-contraction method.

In the League of Nations Memorandum referred to above the oxytocic method is suggested as the official one with the blood pressure method as an accessory. Certain objections are, however, pointed out in regard to the use of decerebrate preparations for purposes of evaluating the strength of post-pituitary preparations (Abel, Hogben and Schlapp).

Action of Infundibular Extracts on the Respiration. Some very characteristic changes in the respiration are induced under the influence of post-pituitary extracts. Houghton and Merrill noted acceleration of the respiration in dogs, while Mummery and Symes observed shallow respiration in dogs and cats following the administration of infundibular extracts. Houssay (1911), Dunan (1911), Fühner (1912) and Pankow (1912) and later Fröhlich and Pick described apneic intervals which follow the intravenous injections of such extracts into unanesthetized as well as into urethane-anesthetized rabbits. These striking respiratory changes do not occur on a second injection. After intravenous administration of 5 to 10 cc. of freshly prepared

pituitary extracts in men Leschke noted periods of apnea. Von den Velden, McKinley and Behrenroth noted no appreciable action in man. Sachs found inconstant results. Abel and his collaborators find with their purified pituitary tartrate respiratory changes similar to those first described by Houssay in 1911. These workers as also Kolls and Geiling compared alterations in respiration produced by the intravenous injection of the purified pituitary tartrate and of pituitary liquid (Armour) into unanesthetized dogs, and the changes produced by the two substances are identical in all respects. The following are the changes noted: after a variable period following the intravenous administration of pituitary tartrate or pituitary liquids the animal pants at intervals. The respiratory rate at these times is often 60 or more per minute. This periodic acceleration may occur early, but is also at times a rather late manifestation. Comparable changes under ether have never been noted in their series of anesthetized animals. Repeated injections of large quantities may stimulate the respiratory rate of a dog under ether, but such quantities of the drug are far in excess of the amount which invariably results in periods of very marked respiratory acceleration in the anesthetized animals. Houssay (1911) also noted that dogs anesthetized with chloralose responded somewhat differently from unanesthetized animals. With subcutaneous injections the increase in respiratory rate is small or is totally absent, and in these cases periodicity in the respirations has not been seen in any of the dogs used by these investigators.

Both Loewi and Meyer and Roberts offer an essentially similar explanation of this apnea following injection of pituitary extract in rabbits, namely, that it is the result of constriction of the vessels of the medulla. The former by administering amyl nitrite with the pituitrin were able to prevent apnea. Roberts, however, presents more direct proof for this view by means of experiments in which he determined the blood pressures simultaneously in the carotid artery and the Circle of Willis. Moreover, by injecting the pituitrin into the carotid he noted that the apnea could occur before the systemic reaction. It must, however, be remembered that these experiments were done under anesthesia and the situation is thus in many respects fundamentally altered. Nevertheless, if one considers the marked lowering of oxygen consumption by the tissues and also the striking peripheral vaso-constriction which takes place, the simplest explanation of the respiratory changes would appear to be along the lines mentioned in Roberts' papers. Some unpublished experiments of

the writer indicate a definite change in the pH of the blood towards the acid side following the intravenous administration of pituitary liquid (Armour) in doses of 1 cc. into unanesthetized dogs, and perhaps this change in acidity may be an added factor in producing the characteristic alterations in the respiration by influencing the respiratory center directly. Infundibular extracts may be of value in relieving acute attacks of asthma.

Action on the Involuntary Musculature, Excluding the Vascular System. Dale (1906) first recorded that post-pituitary extracts stimulate the uterus to increased contraction. This action, which has been verified by all investigators in this field of work, is a very sensitive one, and today the chief method of assay of infundibular extracts is based on its oxytocic action. For this purpose the horn of a virgin guinea pig's uterus is the most suitable. Although many workers have used the oxytocic method with great success, it must be admitted that difficulties may be experienced. Recent studies on the oestrous cycle in the guinea pig reveal a marked variation in excitability of the organ at different periods during the oestrous cycle. In assay work histamine has been generally used as the standard of comparison, but there are objections to the use of this compound. Consequently Smith and McClosky have prepared a dry acetone-extracted infundibular powder to be used as a standard. This powder is finding wide use now and its employment as a standard against which extracts of unknown strength are matched has decidedly improved the technique of the oxytocic method of assay of pituitary extracts. (This powder may be advantageously used as a standard where either the pressor or the diuretic method of standardization is made use of.) It may be appropriate to remark here that the Committee on the Standardization of Drugs, appointed by the League of Nations, has recommended that the oxytocic method of standardization of post-pituitary extracts be the official one and be supplemented by using the blood pressure method.

By the use of the oxytocic method Abel, Rouiller and Geiling were able to demonstrate the extreme potency of their purified pituitary tartrate. Their most potent preparation had an oxytocic value anywhere from 1000 to 1250 times histamine acid phosphate. In other words maximum contraction of the virgin guinea pig's uterus suspended in Tyrode solution was secured in a dilution of pituitary tartrate of 1:15,000,000,000. It should be pointed out here that these investigators in the course of the development of their method of purification

obtained preparations of varying degrees of activity, but in all cases where more than one of the physiological properties of the hormone were utilized for purposes of evaluation, there was found a striking parallelism in potency. That is to say, a preparation of a high oxytocic titer would give a rise of blood pressure or affect the kidney in a corresponding dilution. Furthermore, destruction or injury of the hormone would be reflected in all of the physiological tests employed, thus adducing very strong evidence in support of the unitarian conception of the hormone in the posterior lobe of the hypophysis—the contention of Abel, Rouiller and Geiling.

Clinical uses. Blair Bell and Hick and Hofbauer independently introduced pituitary extracts into clinical obstetrics. Since then this substance has found very wide use, and also much abuse, in obstetrical practice. Blair Bell in his book gives an account of the literature, as does also Houssay in his monograph. Williams in a recent article on the use of extracts of the pituitary gland in obstetrics gives as the three main uses of this drug in practical obstetrics,

> *1*, to stimulate contractions in the second stage of labor when the uterus is relatively atonic, thereby hastening the completion of delivery, or obviating the necessity for the application of forceps (Hofbauer); *2*, to control atonic hemorrhages following the extrusion of the placenta (Blair Bell), and *3*, to induce labor during the last weeks of pregnancy (Hofbauer).

This writer adds further:

> it may be said that the use of pituitary extract in obstetrics should not be considered as harmless, since we are dealing with an extraordinarily potent agent, which is as yet imperfectly standardized. Generally speaking, I regard as somewhat dubious its use in the treatment of prolonged labor even under the most favorable conditions, and as extraordinarily dangerous in the presence of disproportion or of malposition of the child. Its greatest field of usefulness is in the prevention and control of atonic hemorrhage following the third stage of labor, while it is relatively efficient in the induction of labor during the last weeks of pregnancy.

The Alimentary Tract. The action of pituitary extracts on the intestines has been commented upon by a number of observers. Foderà and Pittau and Blair Bell noticed actual defecation following injections of the extract. Blair Bell examined this action further by suspending loops of intestine in Ringer's solution and found that the addition of infundibular extracts to the solution causes a relaxation and cessation of movement, but often this action is preceded by a powerful contraction. Dale independently observed that an isolated loop

of a dog's intestine contracted feebly in the presence of pituitary extracts. Roth in 1917 studied this matter in detail and as a result of his work it appears plain that pituitary extracts contract the rabbit's intestine without exception. The relaxation produced by some extracts is ascribed by him to the presence of chloretone in such extracts. These observations have been fully substantiated by the work of Houssay and his pupils and by Zondek. Bayer and Peter in attempting to explain their results on the isolated intestine which shows, according to them, under the influence of pituitary a diminution in movement followed by a contraction, interpret this dual action as follows: the initial diminution of muscular contractions is due to stimulation of the sympathetic fibers, while the subsequent contraction results from a stimulation of the nerve fibers of Auerbach's plexus. Young's work confirmed the fact that pituitary extracts increase the intestinal tone, especially in the cat, but that large doses are required.

In a recent paper MacDonald studied the action of depressor-free pituitary extracts on isolated strips of intestinal muscle and from his results he is inclined to throw doubt on the commonly held view that the pituitary gland contains a general stimulant of plain muscle fibers. He claims that the intestinal stimulant differs from the other known pituitary principle in being soluble in alcohol and also stable towards boiling alkalies as has been previously shown by Guggenheim. It must be pointed out that this author's tracing shows some stimulation of the intestine, slight to be sure, where pituitary extracts are used. Furthermore, previous investigators have commented on the fact that intestinal muscle is less sensitive than other smooth muscle to pituitary extracts. The presence of depressor substances in commercial preparations must be reckoned with, and some of the stimulating action of such extracts is undoubtedly due to depressor bodies (histamine and others; Abel and Nagayama and Abel and Kubota). In view of MacDonald's results it may be advisable to await further work to settle the question as to the true response of intestinal muscle to the unadulterated autacoid from the posterior lobe of the hypophysis.

Stomach. The results obtained with post-pituitary extracts on the stomach are not concordant. According to Houssay there are four reasons to account for this fact.

1. Improper preparations of the extracts whereby the substance acting on smooth muscle is destroyed.

2. Acidity of the extract which first produces a relaxation of the isolated organ.

3. Preservatives or anesthetics which, like chloretone, for example, inhibit the isolated organ.

4. Individual susceptibility—"neuro-endocrine state."

The present status of our knowledge on this phase of the subject may be said to be as follows: In the case of isolated strips of stomach, or in the organ, in situ, fresh pituitary extracts, free from preservatives, always produce contractions. In Houssay's monograph will be found a complete set of references to the work done on this topic.

Pituitary extracts are now extensively used in therapeutics for bringing about contractions in atonic conditions of the stomach and intestinal muscles, especially following abdominal operations (Blair Bell).

Bladder. It is generally recognized that infundibular extract produces a contraction of the bladder (Frankl-Hochwart and Fröhlich and Blair Bell). Here may be mentioned some interesting unpublished experiments of Macht's which I am privileged to refer to. This investigator using our purified pituitary tartrate on isolated strips of muscle obtained from the trigone and from the fundus of the bladder found both respond in an identical manner to pituitary tartrate, thus pointing to a direct action on the muscle substance, since Young and Macht demonstrated a difference of innervation in the various components of the bladder. They found 1, sympathetic drugs cause contraction of the trigone and relaxation of the fundus muscle; 2, parasympathetic drugs act on the fundus, but have no effect on trigone muscle; 3, "muscle" drugs act in the same way on both trigone and fundus muscle. Pituitary extracts and our purified pituitary tartrate belong to this class. Frankl-Hochwart and Fröhlich and Blair Bell recommend the use of post-pituitary extracts in paresis of the bladder. Hofbauer suggested the use of this extract in puerperal ischuria.

Bronchi. Houssay in 1911 demonstrated that extracts of the posterior lobe, but not of the anterior lobe, produce constriction of the bronchi in the living dog and in curarized guinea pigs, as well as of the isolated strip of bronchial muscle of the dog. Baehr and Pick confirmed this constrictor action by perfusing isolated guinea pig's lungs. Fröhlich and Pick have observed in the rabbit and in the guinea pig that injection of hypophysin produces a broncho-constrictor action due to a stimulation of the vagus. This has been confirmed by Dixon and

Halliburton. It must be pointed out that all these investigators used pituitary extracts containing varying amounts of depressor substance. This fact was brought out by Jackson and Mills who showed that bronchial action in the spinal dog varies greatly with different extracts, and does not depend on the active principle but is apparently to be attributed to the histamine that they contain. Abel and Nagayama point out that infundibular extracts prepared from fresh glands without lengthy boiling or exposure to acids contain only a small amount if any histamine in the free state, while the commercial preparations contain much more of this potent base. Roca in Abel's laboratory studied the relative amounts of depressor and broncho-constrictor substance obtained from the anterior and posterior lobes of the fresh pituitary gland and found that when extracts of both parts of the gland are treated with acid there is formed a chloroform-soluble depressor and broncho-constrictor substance (histamine) which is obtained from the posterior lobe in twenty times the concentration of that formed from the anterior lobe. When, however, the uninjured pressor and oxytocic phosphate "A" prepared by decomposing the "proteid $HgCl_2$-precipitate" of Abel and Nagayama was injected intravenously into a pithed dog, a pure pressor effect and no constriction of the bronchi resulted. Kolls and Geiling confirmed this finding. It is thus clear that the infundibular principle freed from depressor bodies causes no constriction of the bronchi in a pithed dog.

Gall Bladder. Houssay in 1911 found an immediate increase in muscular tonus and accentuation of the rhythmic contraction with post-pituitary extracts. See also Schöndube.

Esophagus. Houssay, Santi and Bottazzi observed contraction while Waddell, Parisot and Mathieu and Bercovitz obtain relaxation with infundibular extracts. Hogben and Hobson found pituitary extracts to be without action on the crop of Aphysia and the pharynx of the aphrodite.

Pupil. Cramer in 1908 reported that infundibulin produces a dilatation of the pupil in the enucleated eye of the frog. This observation has been confirmed by Borchardt, Pal, Bayer, Cattapano, Houssay, Marañon, Mazzei. Recently Pollock found that instillation of pituitary extracts into the eye of a rabbit produces mydriasis in 94 per cent of the cases. This action is even more marked if the cervical sympathetic has been previously sectioned. He found, however, that this hormone introduced intravenously causes hypertension and myosis, owing to excitation of the oculo-motor nerve. But if this nerve has

been previously cut, a marked immediate and transient dilatation occurs. According to this author, pituitary extract exerts its dilator action through the myo-neural junction.

The Diuretic-Antidiuretic Action of Posterior Lobe Extracts. Although the diuretic-antidiuretic property of the infundibular hormone has been very intensively studied by many investigators since 1901 (the year in which Magnus and Schafer first demonstrated the diuretic action of pituitary extracts injected intravenously into anesthetized dogs), and while it is safe to say that the main facts may be regarded as settled, there is as yet no agreement as to the mechanism of the action—whether renal or extrarenal—nor is there unanimity of opinion as to whether the diuretic or antidiuretic action is the real one. Despite this lack of agreement, it seems that the available experimental data, taken collectively, permit of a feasible explanation for the action of the post-pituitary hormone on the renal system; this effect, as will be shown later, may be diuretic or antidiuretic, depending on the conditions to be mentioned. As is natural, experimenters adopted different methods in studying this problem and often used different species of animal, some employed anesthesia, others worked on unanesthetized animals. One set of workers used a small dose, others injected larger quantities. Then again some investigators induced a diuresis in animals before the extract was injected, while others used the normal fasting animal; added to this, certain investigators superimposed an anesthetic on the experimentally produced diuresis. Observations were carried over varying lengths of time—from fifteen to twenty minutes to twenty-four or more hours. Still another variation was introduced—some experimenters produced damage by the use of poisons such as cantharides and uranium in an attempt to determine the seat of action of the infundibular hormone.

These factors, and probably others not mentioned here, must all be considered and are the determinants as to whether the action of the posterior lobe hormone will be diuretic or antidiuretic, or a mixed action of increased flow followed by a stoppage, or vice versa.

Another possibility is that this dual action of posterior lobe extracts may be attributed to two separate bodies, and that the diuretic or antidiuretic effect would predominate, according as to which of the two bodies exerted the more powerful action. Hence it is evident that this point must be satisfactorily disposed of before attempting to offer an explanation of the conditioned diuretic-antidiuretic action. The work of Abel, Rouiller and Geiling seems to remove all doubt

about the diuretic-antidiuretic action of pituitary extracts not being due to a single substance. In a previous section of this review it was pointed out that these investigators have prepared from post-pituitary extracts a highly potent tartrate which possesses all the characteristics of a high-grade extract, including the power to produce either a diuretic effect in the green-fed anesthetized rabbit or an antidiuretic action in patients with diabetes insipidus, and thus there is very little room for doubt that the varying actions on the renal system are due to but one substance which in addition possesses pressor and oxytocic properties.

A review of the literature need not be given here, because it would be merely repeating work well done by several investigators who within the last year have published papers in which the researches of previous workers are discussed. The publications referred to are those of Fromherz, Molitor and Pick, Mackersie, Smith and McClosky, Craig, Stehle and Bourne.

The following facts may be looked upon as definitely established:

1. The injection of small amounts of post-pituitary extracts into anesthetized animals (green-fed rabbits, dogs, cats and guinea pigs) produces a temporary suppression of urinary flow followed by a diuresis of short duration, then either a return to normal flow, or a period of diminished flow (Magnus and Schafer, Schafer and Herring, Dale, Halliburton, Candler and Sikes, Ott and Scott, Hoskins and Means, Pentimalli and Quercia, Römer, Konschegg and Schuster, Myenberg, Oehme, Knowlton and Silberman, Stoland and Korb, Cushny and Lambie, Richards and Plant, Abel, Rouiller and Geiling, Fromherz, Mackersie, Smith and McClosky, Craig, Stehle and Bourne).

Mackersie offers a satisfactory explanation for the brief cessation of urinary flow which follows immediately upon the injection of the extract. He demonstrated this fact by inserting a cannula into the left ureter close to the hilus, as well as one in the bladder. Following each injection of a small dose of pituitary extract there was an immediate increase in flow from the ureter, while a delay of $1\frac{1}{2}$ to 5 minutes occurred in the bladder flow. Molitor and Pick, working on dogs with bladder fistulae, independently of Mackersie, report spasm of the bladder and ureters following injection of pituitary extracts. The initial stoppage in the flow of urine following introduction of the posterior lobe hormone is thus explained as an extra-renal effect.

2. Temporary diuresis occurs after repeated injection of the extract, even though the effect on the blood pressure becomes small, or may even give way to a depressor effect (Schafer and Herring and others).

3. In some cases larger doses when injected into anesthetized animals (notably rabbits) produce an anti-diuretic effect (Beco and Plumier, Konschegg and Schuster, Abel, Rouiller and Geiling, Mackersie).

4. Pituitary extracts in varying doses when injected into normal human beings, into patients with diabetes insipidus, into normal dogs, and into normal rabbits produce an essentially antidiuretic effect (Von den Velden, Farini, Konschegg and Schuster, Motzfeldt, Myenburg, Eisner, Priestley, Jacobson, Garnier and Schulmann, Molitor and Pick, Smith and McClosky, Bagley, Davis, Whitman and Scott).

5. Pituitary extracts introduced into human beings and into unanesthetized animals in which a diuresis has been induced either through ingestion of fluid or by means of diuretics cause a distinct decrease in the output of urine (Houssay, Galan and Negrete, Priestley, Weir, Larson and Rowntree, Molitor and Pick. Underhill and Pack, Fromherz, Smith and McClosky, Stehle and Bourne). Coincident with this diminution in the urinary elimination the blood undergoes dilution, and changes occur in the sodium chloride, urea and uric acid (Hammett, Patten and Suitsu) content of the blood.

The recent contributions of Priestley, Fromherz, Addis, Barnett and Shevky, Smith and McClosky, Starling and Verney, and Stehle and Bourne have added much towards a better understanding of the mechanism involved in the action of pituitary extracts on the kidney.

Priestley, Fromherz, Starling and Verney, Stehle and Bourne report a marked increase in the excretion of chlorides following injections of post-pituitary extracts. This augmented elimination occurs whether the response be increased or decreased elimination of urine.

In viewing the data collectively it seems that the true effect of injecting infundibular extracts into normal experimental animals is an antidiuretic one, while the diuresis observed by most authors is in a great measure a conditioned effect, with anesthesia and previous feeding probably playing the chief rôles. It must, however, be admitted that dosage is an important determining factor, for even in the anesthetized animal larger doses may have an antidiuretic action also.

It is in respect to the mechanism whereby an increased flow or a diminished output of urine is brought about that investigators fail to agree. Most authors, however, agree that the diuretic-antidiuretic action of the infundibular hormone is a renal affair although Weir, Larson and Rowntree, Underhill and Pack, and Molitor and Pick

assert that the hormonal effect is extra-renal. Weir, Larson and Rowntree and Molitor and Pick believe the effect of the pituitary extracts is through some regulatory function on the water depots. The last-named workers produced polyuria by injury to the kidney of dogs with cantharidin and uranium salts and observed no antidiuretic action with pituitary extract. In addition vaso-dilator drugs, such as nitrites, had no effect on the antidiuresis produced in normal dogs by pituitrin, neither did the exclusion of the liver (by an Eck fistula) from the circulation but diuresis was restored by administration of sodium chloride, glucose and urea which, they state, probably act by removing the excess of water from the tissues in which it has been stored and causing its rapid excretion by the kidneys. While these findings are interesting it must be admitted that deductions drawn from experiments in which kidney damage is produced by the use of cantharidin, uranium salts or tartrates are open to criticism, and can be explained satisfactorily on a renal basis without looking for hypothetical extra-renal causes, such as interference with the regulation of the water-balance in the tissues. It may perhaps be well, however, to leave the matter of disturbed water-balance under pituitary influence as an open question for the present.

Underhill and Pack believe that the hydremic and antidiuretic action of pituitary extracts are probably due to some as yet unexplained action on capillary permeability.

The diuresis produced in anesthetized animals after injection of infundibular extracts has been explained in several ways by different workers. Knowlton and Silverman and Cushny and Lambie regard the diuresis as being due to an increased flow of blood through the kidney, while Starling and Verney state that this action cannot be explained through its vascular effect. Richards and Plant explain the diuretic effect of minute doses of pituitrin as due to increased glomerular pressure arising from constriction of the efferent vessels and hence increased urine formation.

The results obtained by the above mentioned investigators seem to indicate that the diuretic effect may be due 1, to increased flow of blood through the kidney, or 2, to increased glomerular pressure. Both conditions can bring about a diuresis. Added to this, there is increased sodium chloride excretion which may draw more fluid through an osmotic effect. Concentration of the glomerular fluid by the epithelial cells of the tubules is prevented either as a result of the direct

action of the anesthetic on these structures or also through the increased amount of sodium chloride present in the lumen of the tubules. The latter would account for the diuresis produced in those instances where no anesthetic was used. Larger doses of pituitary extract have also an antidiuretic effect when injected into anesthetized animals. In such cases the explanation is that the pituitary extract is present in sufficient concentration and the consequent stimulation of the tubular cells (the main seat of action of the hormone) overcomes the inhibitory action of the anesthetic.

The antidiuretic effect of pituitary extracts in human beings or in normal animals, or in cases where a diuresis is induced by previous administration of fluid, may be explained on the basis of a specific stimulation by the hormone of the tubular cells whose concentrating action will be speeded up so as to remove the fluid from the glomerular filtrate at a rate in excess of its normal rate. Owing to the presence of the sodium chloride and other electrolytes a balance is struck, the net result being an antidiuretic effect in the presence of the hormone which exercises a specific stimulatory influence on the epithelial cells of the renal tubules. The blood dilution in such cases would follow as a result of increased reabsorption of fluid.

The antidiuretic action of the infundibular hormone is in all likelihood its normal action; the diuresis obtained in certain cases is a conditioned action brought about by anesthetics and other factors. For this reason, probably, considerable experimentation will have to be done in the future when the hormone will be available in a chemically pure form, such as epinephrin is at present. Hence the explanation here suggested and discussed must be regarded as tentative and subject to modification as results come forth with improved methods and purer preparations.

Account may be taken here of the work of Rees who showed that pituitary extracts subcutaneously introduced into anesthetized dogs and cats caused a delay in the absorption of water from the small intestine. This action was thought by him to be in part responsible for the antidiuretic action; to substantiate this view he injected physiological salt solution intravenously into dogs and found that the diuresis was not prevented by these extracts. This author found such extracts when introduced directly into the lumen of the small intestine in dogs and cats will cause a delay in the absorption of water from that organ. The delay in such cases is about the same as that

caused when the extracts are injected subcutaneously. Further he showed that infundibular extracts introduced into the lumen of the stomach prevented absorption of water from the small intestine.

Von den Velden and Farini first used pituitary extracts in the treatment of diabetes insipidus and since then the efficacy of such extracts in alleviating the symptoms of this disease has been so abundantly discussed that we need but refer to it in passing. Rowntree has recently written a review on the clinical aspects of diabetes insipidus and its therapeutic management. Abel and Geiling have had the opportunity of submitting their purified pituitary tartrate to a clinical test on four cases of primary diabetes insipidus and it was found that this salt was as effective as a high grade commercial extract, dose for dose, in checking the excessive urinary output and allaying the incessant thirst.

Mackersie recommends the diuretic and antidiuretic effects on the rabbit as a means of estimating the potency of post-pituitary extracts. Pick and co-workers suggest that the antidiuretic action on the dog with a bladder fistula be used as a means of standardizing infundibular extracts, since they state that the antidiuretic action goes completely parallel with the oxytocic property, which is the most generally used method of standardizing such extracts.

Action of Pituitary Extracts on the Glandular System. A. Mammary gland. In 1911 Ott and Scott found that the injection of pituitary extracts into lactating goats had a marked galactogogic action. Since then numerous workers have confirmed this finding (Mackensie, Hammond, Schafer and Mackenzie and Simpson and Hill, Houssay, Gavin, Gaines, Blair Bell) for practically all available species of lactating animals. A good and complete review of this phase of the subject is given by Houssay in his monograph on the hypophysis and the supplement to it. Suffice it to state here that there is no doubt as to the fact that the injection of infundibular extracts into lactating women and experimental animals causes a surprisingly large increase in the lacteal secretion. This property is confined to the posterior lobe alone; the anterior lobe extracts have no such action. The milk secreted after pituitary administration is usually rich in fat content. Investigators, however, disagree as to the seat of action. Hammond, Houssay and Hill, and Simpson regard the action of the hormone as being a direct stimulation of the mammary secretory epithelium. Schafer, Gaines and Blair Bell oppose this view, and contend that the increased lacteal secretion is due to a con-

traction of the smooth muscle fibers of the alveoli. The augmented milk secretion seems to have little to do with the increase in blood pressure produced by the injection of post-pituitary extracts, for while repeated injections of this material have no effect on the blood pressure, they nevertheless still cause an increased milk flow, although, it must be said, in diminishing amounts as compared with the stimulation following the initial injection.

In this connection it may be interesting to note that Houssay claims for the galactogogic property of the infundibular hormone a specificity and delicacy exceeding that of any of the other physiological actions of the principle mentioned.

Pituitary extracts are of little value therapeutically as a galactogogue when the secretion of milk is deficient. (Heaney and others.)

B. Salivary secretion. The work of Oliver and Schafer, Dale, Hochwart and Fröhlich, Solem and Lommen and Houssay shows that the pituitary extracts do not increase salivary secretion although if the flow of saliva has been increased by a previous injection of pilocarpine or a stimulation of the chorda tympani injection of infundibular extracts tends to diminish the flow. Observations repeatedly made in this laboratory on unanesthetized dogs injected with either pituitary liquid or our pituitary tartrate are in agreement with the above findings, except that we have noted a definite diminution of salivary flow after large doses administered intravenously. The animal's mouth becomes quite dry and parched.

Gorke and Delock and Weir, Larson and Rowntree find that in cases of diabetes insipidus the injection of pituitary extracts produces an increase in salivary secretion.

C. The gastric, pancreatic and biliary (Alpern) secretions are apparently not increased under the influence of properly prepared infundibular extracts (Pal). Any increased secretory activity would in all probability be due to the presence of varying amounts of histamine. For a complete review of the literature, reference may be made to Houssay's monograph.

D. Lymph secretion. Meyer and Meyer-Bish observed that the injection of pituglandol diminishes by about one-third the quantity of lymph in a dog. The albumin content, however, is increased.

The Action of the Posterior Lobe Hormone on the Pigmentary System of Lower Vertebrates. From the experimental work of Adler, Smith, Allen, Spaeth, Atwell, Swingle, Houssay and his co-workers, Huxley, Hogben and Winton, the fact stands out clearly

that the pigmentary system in the lower vertebrates is specifically under the control of the internal secretion from the posterior portion (neural lobe and pars intermedia) of the hypophysis. Smith and Allen independently removed the buccal anlage from tadpoles, and, among other disturbances previously discussed, they found a striking alteration in the pigmentary system; the animals turned silvery white —they became "albinos." There is some difference of opinion among investigators as to the way in which this change in color comes about. The opinion of Smith, Allen and Atwell will be given here in the briefest form without comment. Smith attributed the albinism to a reduction in the number of the melanophores in the epidermis and to a diminution of pigment granules in these melanophores. Allen on the contrary holds that the changed color of the hypophysectomized tadpoles is due to the migration of the epidermal pigment cells to deeper positions, and also to a contraction of the pigment cells in all parts of the body. He states that "there is no disappearance and bleaching of pigment granules as asserted by Smith." Both investigators agree that the xantholeucophores are expanded. Atwell, adopting the same technique as Smith and Allen for removing the epithelial portion of the hypophysis in tadpoles, studied pieces of entire skin of such hypophysectomized animals, instead of sections such as, he asserts, were used by Allen and Smith and he concludes that the change in color following hypophysectomy in frog larvae is to be attributed to a contraction of the sub-epidermal melanophores, and "only secondarily is it due to a loss of pigment granules from certain of the epidermal melanophores, and to a possible migration or loss of other epidermal melanophores."

The first ablation experiments of Allen and Smith were instrumental in proving that the changes in the pigment cells were caused by a removal of the hypophysis without specifying which particular portion of the gland was responsible for the alterations produced.

The chain of evidence on which rests the fact that the pigmentary system of amphibians is under the control of the post-pituitary secretion may be summarized as follows.

Albinism produced in tadpoles by removal of the buccal anlage *disappears:*

a. When the pituitary gland of adult frogs is engrafted into such silvery hypophysectomized tadpoles (Allen).

b. When silvery tadpoles are placed in dilute extracts of *pars intermedia* (Atwell).

c. When silvery tadpoles are engrafted with *pars intermedia* (Allen, Swingle).

d. When pars intermedia extracts are injected into albino tadpoles (Smith and Smith).

e. When silvery tadpoles are fed pars posterior (plus pars intermedia) (Smith).

Confirmatory evidence was furnished by the work of Hogben and Winton who injected posterior lobe extracts into adult frogs (R. temporaria) rendered light colored by exposure to bright light for a few hours to insure maximal contraction of the melanophores. Such animals when injected with very minute amounts of posterior lobe extracts rapidly turned dark. They, in addition, made extracts of the pituitary gland of the rabbit, duck, lizard (Lacerta viridis), codfish and the frog, and observed that such extracts when injected into pale frogs would cause them to darken in color. In this way they showed that the melanophore stimulant is present in the pituitary gland of various classes of vertebrates. This principle was not found in the subneural gland of the Truncates. The results of Hogben and Winton were verified by Houssay and Ungar who also used paled frogs and made extracts of the hypophysis of a very much larger variety of animals (man, ox, guinea pig, horse, sheep, goat, pig, dog, cat, rabbit, white rat, chick, duck, dove, reptiles (turtle, alligator, Lachesis altematus), toads, frogs and fishes). Houssay and Ungar call special attention to the fact that the melanophore-expanding principle is obtained from the skate, a fish in which, it is claimed, the neural lobe is entirely lacking, or is only rudimentary at best, and from the hypophysis of which no pressor extract can be obtained (Herring, Houssay).

Huxley and Hogben noticed that the skin of Urodele larvae become dark after pituitary injection. Millot injected pituitary extract into albino Urodele and noticed some darkening for one hour and thereafter the animals became pale again. Hogben removed the anterior lobe of the hypophysis from adult frogs and observed no notable change in the color of such animals, but removal of both lobes resulted in pallor some hours after the operation. The pale color in such hypophysectomized animals could not be restored by subjecting them to an environment which would induce darkening in normal animals. The administration, however, of posterior lobe extracts to frogs and axolotls rendered pale through ablation of the pituitary gland was effective in darkening such animals which again regain their characteristic pallor even when kept under conditions conducive to the pro-

duction of the dark color in normal animals. Houssay's experiments are in accord with those mentioned above.

The color changes in amphibians induced by the administration of extracts of the posterior portion of the hypophysis are independent of environmental factors and are *specifically* due to the hormone in the pars intermedia and pars posterior (Hogben and Winton, and Houssay and Ungar).

In this connection it may be of interest to call attention to the fact that administration of pineal gland substance to darkened anuran larvae will cause them to turn silvery like tadpoles from which the hypophysis has been removed. McCord and F. P. Allen demonstrated that the mammalian pineal gland when administered by various routes to anuran larvae will bring about a temporary contraction of the subepidermal melanophores, producing the silvery appearance noted in hypophysectomized tadpoles. Swingle confirmed the above results by transplanting the pineal gland of reptiles (Chelonia) into darkly pigmented larvae of Rana clamitans and noted that within thirty to forty minutes following implantation of the gland the larvae assumed a distinctly lighter color which persisted several hours. Similar results were obtained when mammalian pineal gland in the form of pills was injected into the body cavity of dark larvae of the same species as used above.

The consensus of opinion among workers in this field appears to be that the pigmentary system in amphibia is under direct hormonal control, and by diverse procedures investigators have shown that this autacoid regulation is through a direct action on the pigment cells and not through any nervous intervention. Smith demonstrated this by reciprocal skin grafts, between silvery tadpoles and normal ones. Hogben and Winton established the fact by the process of elimination with the aid of various drugs and physical agents and they also point out the local action in isolated limbs and pieces of skin. Houssay and Ungar also proved the point by using an even larger series of drugs and physical agents than did Hogben and Winton. In addition Houssay and Ungar demonstrated that the melanophore-expanding autacoid was carried by the blood. Sectioning of the sciatic does not modify the blackening of the corresponding foot produced by the injection of hypophyseal extracts (pars intermedia and pars nervosa). Ligature of the muscle, but leaving the nerve free, prevents change in color of the corresponding foot, while the rest of the animal blackens. Krogh and Rehberg showed that in a foot perfused with Ringer's solu-

tion the capillaries dilate and the melanophores contract. Addition of the pituitary extract prevents these changes. Hogben and Winton, Houssay and Ungar and Fenn comment upon the extreme delicacy of the melanophore response to infundibular extracts in amphibians and suggest it as another possible test for determining the potency of various extracts of the posterior lobe of the pituitary gland. Spaeth found that the pigment cells in the scales of the killiefish (Fundulus heteroclitus) contract under the influence of post-pituitary extracts. He suggests this as a possible method for standardizing infundibular extracts.

Gianferrari observed that certain species of salmon (Salmo fario) when fed on hypophysis become decolorized. Hogben obtained no darkening of the skin in four pale chameleons injected with 0.5 cc. of 1 per cent infundin. Houssay and Ungar injected very large doses (28 to 40 cc.) of 1 per cent decoctions of the bovine posterior lobe of the hypophysis into two alligators weighing 1200 and 1500 grams and 25 to 30 cc. of this extract into two snakes, weighing 205 to 260 grams, with very little effect; only after 24 hours was there a doubtful intensification of the color in the spots of both species. They injected intraperitoneally varying doses into fishes of different weights and found no change of color in the majority of experiments and in no case was there positive darkening. Scales and pieces of the skin of the yellow catfish immersed in pituitary extracts showed no change in color or in the microscopic appearance of the melanophores.

Unclassified Facts Concerning Post-Pituitary Extracts. Schafer and Magnus showed that infundibular extracts cause a contraction of the spleen. Dale has confirmed this observation. de Boer and Carroll found that the diminution in splenic volume following the intravenous injection of pituitrin into the intact animal (cat) is due solely to vaso-constriction within the organ, and not to a contraction of the muscle of the splenic capsule.

Dale found that the urine of a cat injected with pituitary extracts had a pressor action when tested on another cat, thus indicating that the active principle is, in part at least, excreted unchanged in the urine.

Mautner and Pick in attempting to explain the differences in blood pressure response due to pituitary extracts in various species of animals made studies on dogs, cats and rabbits, by determining the volume changes in the liver with a specially constructed oncometer and at the same time recorded the blood pressure changes. They noted a definite diminution in liver volume in dogs and cats, as also in rabbits following

intravenous administration of pituitrin. At the same time there was a sharp fall in blood pressure followed by an insignificant rise in the case of dogs and cats while in the rabbits there was a steep rise of blood pressure. This difference in blood pressure action on the various species is thought by these authors to be due to the presence in pituitrin of histamine-like depressor substances which in dogs and cats effect a constriction of the liver veins and in turn cause imperfect filling of the heart, resulting in a fall of pressure. This does not happen in the case of rabbits. The pressor action in dogs and cats wears off with repeated action while the depressor effect remains unchanged.

Lamson and his co-workers have found that pituitrin causes a great prolongation of the fall in hemoglobin percentage following the intravenous infusion of salt solution in contradistinction to the effect of epinephrin and histamine which decrease it and that pituitrin would overcome the action of either epinephrin or histamine. At first sight this would appear to mean a retention of fluid in the circulation, but they have shown that a similar action could be obtained by ligation of the hepatic artery which indicated that the prolonged hemoglobin depression might equally well be due to a storing of red cells in the liver. Evidence of such action under different conditions has been previously given by Lamson.

Yoshimoto finds that extracts of the pituitary have no effect on the conductivity of motor nerves.

Rogers destroyed the thalamus in pigeons and found that pituitary extract in such animals causes a diuresis and increase in body temperature. This effect is not seen in normal birds, unless they have been deprived of water and kept in a high dry external temperature. Normal pigeons counteract the injection of pituitary extracts by hyperpnea, flattening of the feathers.

Nogaki observed a lessened vascular response in the case of hypophysectomized frogs perfused with Ringer's solution to which pituitary extract or adrenaline has been added.

Basal Metabolism and the Pituitary Body. Within the last year three monographs (duBois, King and McCann) and an extensive review article by Boothby and Sandiford have been published on basal metabolism and in them the relation of the pituitary gland to metabolism is discussed, consequently here we shall take up only a few of the important points and attempt to give a summarized statement of the present status of our knowledge of the influence of the pituitary gland on metabolism.

Prior to the studies of Benedict and Homans most of the metabolic studies were made upon normal subjects fed or injected with pituitary preparations. These workers, however, prompted by the earlier work of Crowe, Cushing and Homans who described peculiar changes in the temperature of their hypophysectomized dogs in the last stages of cachexia hypophyseopriva, made careful and extensive studies of the metabolic changes in dogs from which the pituitary had been removed.

Their results are summarized under six headings—body weight, body temperature, pulse, respiration, total metabolism, development and general appearance. All these body processes are reduced, indicating a general lowering of all the normal body functions; some gain in weight, however, was noted, due to a deposition of fat. Winton and Hogben noted a lowering in the rate of carbon dioxide production in hypophysectomized frogs. These authors are inclined to attribute this to anterior lobe deficiency.

Other investigators have injected pituitary extracts from the different portions of the gland and obtained variable results. Weiss and Reiss studied the gaseous metabolism in starved and in fed rabbits and found a lowering of the metabolism and increased utilization of carbohydrates after injection of pituitrin. A number of workers (Bernstein and Falta, Tierney, Snell, Ford and Rowntree, Boothby and Sandiford, Plaut, Boothby, McKinlay, Labbé, Stévenin and van Bogaert, Pareja and Zlaczower) have made metabolism studies on normal human subjects injected with pituitary extracts, or on patients with various diseased conditions of the hypophysis, and it may be said that there is very considerable variation in the results obtained. This fact is not surprising if we consider the great differences in potency of commercial extracts, and that investigators did not all use the same dose. Furthermore there must have been wide variations in the severity of the diseased conditions among the patients used. In general, it would seem that in many cases of hyperpituitarism there was an increase in the basal metabolic rate, while in a number of patients with a hypofunctional state of the hypophysis (Fröhlich's syndrome) there was a lowering of the basal metabolism. The results, however, are by no means consistent. It is important to remember that there is a very striking interdependence between the various glands of internal secretion, and that disturbances in metabolism in diseases associated with pathological conditions of the hypophysis may not necessarily be ascribable to that gland, but may be due to an alteration in the function of another gland (thyroid, or adrenals) resulting from a diseased pituitary body.

INTERRELATIONSHIP OF THE PITUITARY BODY WITH OTHER ENDOCRINE GLANDS. A fact of the utmost importance is that there exists a very close interdependence between the various glands of internal secretion; an excessive or diminished function of one of them is almost invariably reflected in one or more of the members of the endocrine system either in their structures, or in the physiological functions controlled by the hormones elaborated by them. The facts are already many and their number is growing daily, and with each discovery new light is brought on this obscure but all-important phase of biology. We shall here take up only the more important instances in which the hypophysis is involved.

1. Castration causes an increase in weight and volume and also characteristic histological changes in the hypophysis (Fichera, Izumi).

2. The pituitary gland is enlarged in the progeny of thyroidectomized and adrenalectomized rabbits (Seitz).

3. During pregnancy the anterior lobe of the hypophysis hypertrophies (Erdheim and Stumme).

4. Destruction of the hypophysis by disease or experimentally or its surgical removal or sectioning of the infundibular stalk results in the development of Fröhlich's syndrome (Paulesco, Cushing and his associates, Aschner, Blair Bell, Biedl, Smith, Walker and Graeser).

5. Hypophysectomized tadpoles show characteristic changes in growth, in the pigmentary system and in the thyroid and interrenal bodies, and they do not metamorphose (Smith, Allen and others).

6. Peritoneal injection of anterior lobe extracts into normal rats produces gigantism and impairs or prevents the growth and maturation of ova, so that oestrus is infrequent or absent (Evans and coworkers).

7. Injection of anterior lobe extracts into normal and thyroidless tadpoles accelerates their growth rate and increases the depth of pigmentation (Smith and Smith). Gland transplantation experiments show similar results (Allen and Swingle).

8. Izumi finds that removal of the parathyroids in rats and cats affects some of the cells in the pars anterior; in the rats the hypophysis is diminished in size, while in the cats it is hypertrophied through proliferation of the cells of the pars intermedia.

9. Thyroidectomy in cats causes hypertrophy of the anterior lobe and lobus pedimentoris.

We may in conclusion refer to only a few of the numerous instances of the mutually antagonistic action of pituitrin and other glandular

extracts. Thus, pituitary extracts inhibit epinephrin hyperglycemia, and recently Burn, as also Moehlig and Ainslee, showed that the simultaneous injection of pituitary extracts and insulin into starved rabbits prevents the hypoglycemia which invariably follows from the injection of the latter substance alone.

However important the clinical aspects and the pathology of a diseased state of the hypophysis may be, a discussion of them here is omitted as lying outside the field of the writer's personal studies.

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THE PHYSIOLOGY OF THE CEREBELLUM

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The doctrine of cerebellar function prevailing at the present time is the outcome of efforts to construct a logical hypothesis in accordance with the inferences derived from a vast number of experimental, clinical and morphological studies. The importance of anatomical considerations in such a theory of neural activities is well exemplified by the cerebellum: here, the neuronal relationships, the origin and destination of fibre-tracts as well as the gross topography of the organ have served not only as a basis for the interpretation of cerebellar phenomena but also as a place of departure for many lines of fruitful research.

MORPHOLOGY OF THE CEREBELLUM. In the following brief outline of cerebellar morphology attention will be directed to the facts which are of special significance in relation to functional activities; the subject is treated extensively in the valuable contributions of Bolk (1906), Herrick (1914, 1922, 1924), Ingvar (1918, 1923), Ranson (1921), Tilney and Riley (1921), Kappers (1921), Tilney (1923) and Marburg (1923).

The type of cerebellum which was, in the view of Herrick, ancestral to that of higher vertebrates is found in the Petromyzontes. It is a plate developed as a direct continuation of the octavolateral area, thus demonstrating a fundamental principle of cerebellar construction; its neuronal elements are of the nature of Purkinje and granule cells. A spinocerebellar tract, though probably present, has not been positively demonstrated.

Proceeding upwards in the animal series the essential nature of the cerebellum becomes apparent as a correlation centre for vestibulo- and spinocerebellar impulses. For instance, in the elasmobranchs and teleosts the termination of the spinocerebellar fibres becomes strongly developed and also the ending-place of the vestibulolateral fibres. In the amphibia the receptive centres for the spinocerebellar fibres are in the median body, comparable to the vermis of higher forms, while the end-stations of the vestibulolateral fibres are in the auricular lobe,

the equivalent of the lobus floccularis of the mammalian cerebellum; these two subdivisions form by fusion a single organ, the dual nature of which is thus revealed. Owing to the disappearance of the lateral line fibres in adult frogs the auricular lobe comes predominantly under the influence of the vestibular fibres.

The cerebellum of reptiles shows a great development as compared with that of amphibia, though the auricles are reduced, owing to the absence of lateral line organs. In the large cerebellum of the crocodile we meet, for the first time, a division into lobus anterior, lobus medius and lobus posterior (Ingvar); the lobus posterior is continued into the auricles, which enclose the lateral recesses. The spinocerebellar fibres go principally to the lobus anterior, the vestibular fibres chiefly or exclusively to the lobus medius and lobus posterior.

In the cerebellum of the bird there is a clear division into the lobus anterior, lobus medius and lobus posterior, the division being effected by fissures homologous with the sulcus primarius and sulcus praepyramidalis of the mammalian cerebellum. The auricles, formed as lateral extensions of the lobus posterior, are equivalent to the lobi flocculares of mammals. The majority of the spinocerebellar fibres end in the lobus anterior, some also in the pyramid and uvule of the lobus posterior. Vestibulocerebellar fibres are distributed to the auricles and to the caudal part of the cerebellum. The cerebellofugal paths are comparable to those of mammals.

The cerbellum of man is developed from the portions of the metencephalic alar laminae, considered to be enlargements of the vestibular nuclei, its relationship to the eighth nerve thus being exemplified as in lower forms. While resembling at an early stage the adult amphibian cerebellum, it soon shows a subdivision into the lobus anterior, lobus medius and lobus posterior (Ingvar), the sulcus primarius and sulcus praepyramidalis effecting this partition. In figure 1 is shown the application of Ingvar's schema to the cerebella of reptiles, birds and mammals.

The tripartite division of the cerebellum just mentioned in the case of man and already referred to in the crocodile and bird was adopted by Ingvar, who aimed at a rational partition of the organ on principles of comparative anatomy. The nomenclature adopted first of all for the human cerebellum and afterwards transferred to that of other forms is unsatisfactory from many standpoints, and this applies particularly to the differentiation into "vermis" and "hemispheres." These terms, though lacking adequate justification from embryology

or phylogeny, have, through extensive use, acquired some value as descriptive terms and will, at times, be used in this way in the course of this article. Investigators into cerebellar function have, unfortunately, expressed their results in reference to various systems of nomenclature. It is impossible to review these here; instead Ingvar's mode of division will be outlined shortly, since it is based on sounder principles than those previously propounded; constituting as it does a development, though with important modifications, of the views of

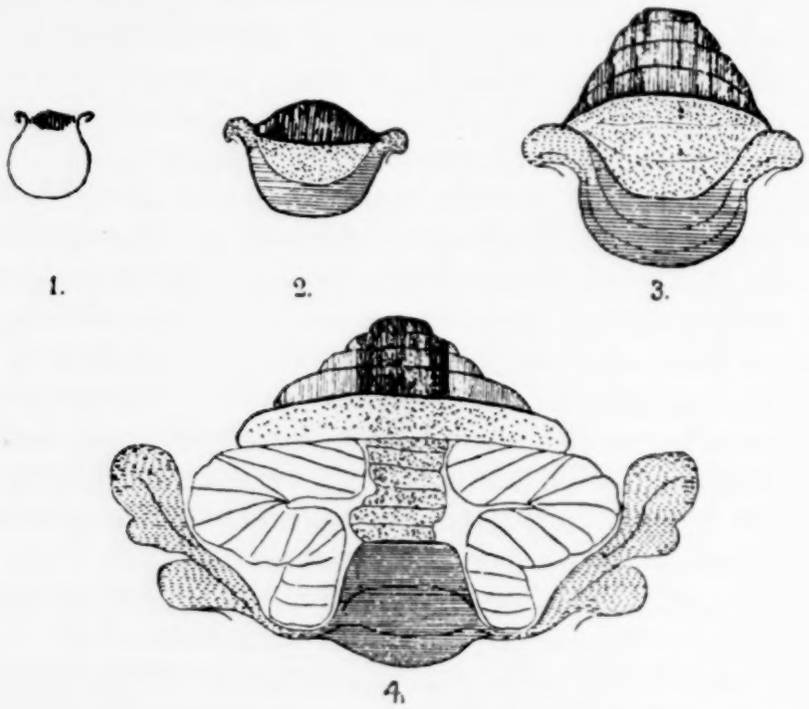

Fig. 1. Schematic representation of the cerebellum of *1*, lizard; *2*, crocodile; *3*, bird; *4*, mammal. Vertical lines, lobus anterior; horizontal lines, lobus posterior; interrupted horizontal lines, lobus floccularis of lobus posterior; stippling, lobus medius; white, lobus ansoparamedianus. (Ingvar.)

earlier writers, especially Elliot Smith (1902) and Bolk (1906), it embraces many terms previously in use. Special terms used by experimenters will, when necessary, be related to better known designations.

In Ingvar's schema of the mammalian cerebellum due emphasis is laid on the importance of the sulcus primarius as a boundary mark, a fact already stressed by Elliot Smith and Bolk; thus there arises a new principle for subdividing the cerebellum, namely, in a transverse instead of in a longitudinal direction. In front of the sulcus primarius is the lobus anterior, which shows no division into "vermis" and "hemi-

spheres." Of the four lobules composing the lobus anterior the first corresponds to the lingula, the second to the lobulus centralis, the third and fourth together to the culmen. To the lobus anterior belong the nuclei fastigii.

The lobus medius Ingvar differentiates between the sulcus primarius in front and the sulcus praepyramidalis behind; in this he differs from Bolk, who designates as lobus posterior everything behind the sulcus primarius, the "vermis" portion behind the lobulus simplex being called by Bolk the "lobulus medianus posterior;" since several investigators have used this latter term in Bolk's sense it seems wise to define it in this place. The lobus medius (Ingvar) consists from before backwards, in the first place, of the lobulus simplex (equals medially the declive, Ranson), which, like the lobus anterior, shows no differentiation into median and lateral parts. Behind the lobulus simplex are found medially the lobulus medius medianus (or lobulus C_2 of Bolk or "S" of Rynberk or approximately the tuber, Ranson) and laterally the lobuli ansiformisparamediani or lobuli ansoparamediani. The lobuli ansoparamediani are new mammalian acquisitions, which develop, obviously, in association with the cerebropontine tracts, which first make their appearance in mammals.

The lobus posterior (Ingvar) lying behind the sulcus praepyramidalis includes the pyramid, uvula and nodule (referred to collectively by Ingvar as the lobulus posterior medianus) and the lateral-lying lobus floccularis or lobus flocculi, which includes the flocculus and paraflocculus. These medial and lateral parts are united by means of the velum medullare posterius, a bond of union which has suffered thinning-out in consequence of the backward growth of the lobulus paramedianus in higher mammals.

Extending his nomenclature to the human cerebellum (fig. 2), Ingvar finds it divisible into the lobus anterior, lobus medius and lobus posterior. Again we find the absence of differentiation between the the median and lateral parts of the lobus anterior and the lobulus simplex (or declive, Ranson). Comparably with the lower mammals, the lobulus ansoparamedianus appears as a structure developed independently of the lobulus medius medianus. The tonsil, regarded by Ingvar as the equivalent of the paramedian lobule, is a direct continuation of the biventral lobule and has undergone a rotation *in loco*. The lobus posterior is composed of the same elements as in lower mammals, namely, the pyramid, uvula and nodule connected by the velum medullare posterius with the lobus floccularis, the latter including the floccu-

lus and the vestigial paraflocculus. The attenuation of the velum medullare posterius is dependent on the same factors as in simpler forms, i.e., the extension backwards of the tonsil and its insinuation between the lobulus posterior medianus and the lobus floccularis.

CEREBELLAR CONNECTIONS. The cerebellum is constituted of a group of reflex centres which, in response to streams of proprioceptive impulses from the labryinths and the body generally, discharge motor influences which regulate the postural tone of the muscles of the body. Fibres

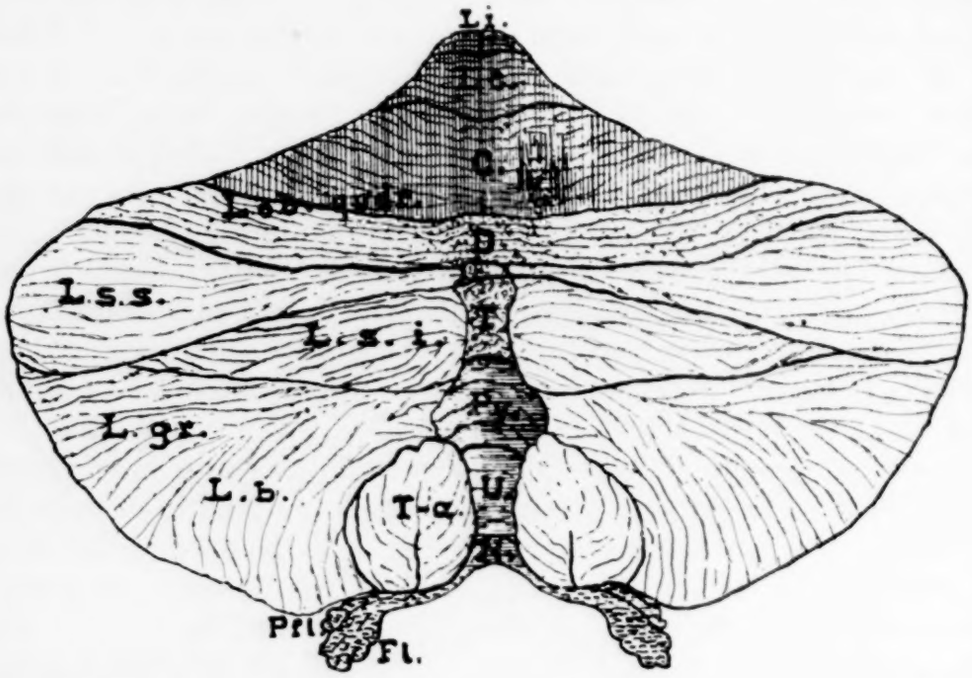

Fig. 2. Diagram showing Ingvar's schema applied to the human cerebellum, the subdivisions being depicted as in figure 1. Lettering in reference to usual nomenclature. (Ingvar.)

of the vestibular nerve reach the cerebellum in part directly and in part indirectly, i.e., after interruption in the vestibular nuclei. Passing through the restiform body both direct and indirect vestibulocerebellar fibres terminate in the nucleus fastigii; as well, the direct fibres are distributed to cortical areas in the lingula (of lobus anterior) and the uvula, nodule and flocculus (of lobus posterior). These lobules, which lie in close proximity to the vestibular nuclei, Ingvar regards as phylogenetically the oldest parts of the cerebellum: he calls them the basal or "vestibular storey."

From the proprioceptors of muscles, possibly also from those of

joints and tendons (Marburg, 1904; Bing, 1906), impulses are conveyed through the dorsal spinocerebellar tract, which enters the cerebellum through the restiform body, and through the ventral spinocerebellar tract, which reaches the cerebellum via the brachium conjunctivum. Ingvar found that the greater number of fibres of both dorsal and ventral tracts end, after decussation, in the cortex of the lobulus centralis and culmen (of lobus anterior); some fibres of the dorsal tract also go to the lobulus simplex (of lobus medius) and to the pyramid and contralateral paraflocculus (of lobus posterior). These locations Ingvar calls the intermediate or "spinal storey" of the cere-

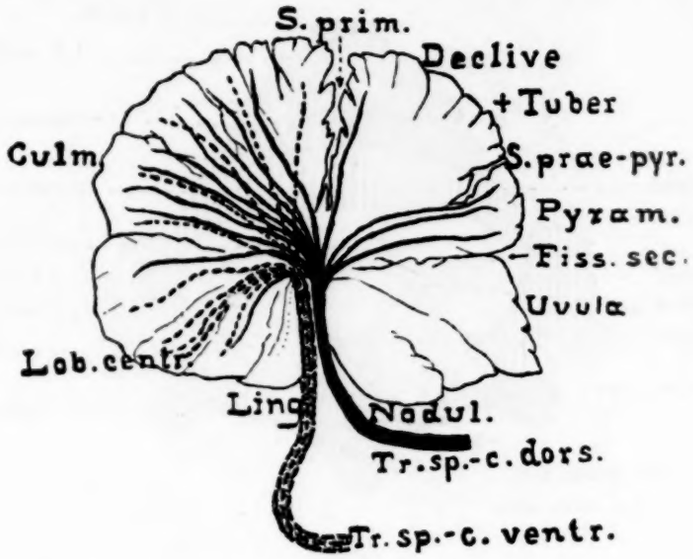

Fig. 3. Terminations of spinocerebellar fibres in the cerebellar cortex of the cat. (Ingvar.)

bellum. Fibres originating in the trigeminal nerve also reach the cerebellum with the dorsal spinocerebellar tract and the tractus nucleocerebellaris. These facts are illustrated in figures 3 and 4. The inferior olive, a receptive nucleus for spinal and possibly thalamic fibres, sends the tractus olivo-cerebellaris via the opposite restiform body to the median and lateral parts of the cerebellar cortex.

Impulses are transmitted from the association centres and motor areas in the cerebral cortex to the nuclei pontis respectively by cortico-pontine tracts and by collaterals from the cortico-spinal tracts. These impulses are then conveyed by the brachium pontis to the median and lateral parts of the cerebellum, the lobulus ansoparamedianus being included.

With regard to the synaptic relationships of these cerebellopetal fibres differences of opinion exist. Thus Cajal (1911) holds that those of the brachium pontis end as climbing fibres around the dendrites of the Purkinje cells, while those of the restiform body terminate as mossy fibres in relation to the dendrites of the granule cells. On the other hand, Jelgersma (1917) maintains that the pontine and olivary fibres end as mossy fibres while the vestibular, spinal and trigeminal fibres end as climbing fibres.

Whichever of these views may prove to be correct it is clear that complicated reflex arcs of various kinds are established, whereby, in

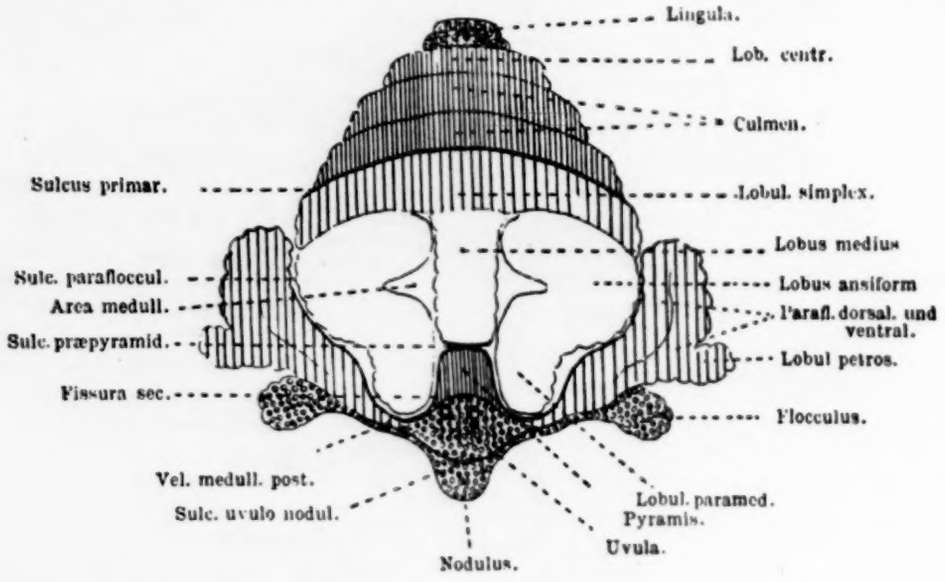

Fig. 4. Schematic representation of regions of termination of direct vestibulocerebellar fibres (small circles) and spinocerebellar fibres (vertical lines) in the cerebellar cortex. (Ingvar.)

response to the afferent, proprioceptive impulses, tonic motor effects are released through the cerebellofugal paths, the main features of which will now be described.

The Purkinje cells, the axons of which terminate in the intracerebellar nuclei, may be conceived as playing an important rôle in discharging the tonic influences just mentioned. While the axons of these cells proceed to the various nuclei from most parts of the cortex, the median cortex is mainly in relation with the medial nuclei and the lateral cortex with the lateral nuclei (fig. 5).

Axons from the nucleus dentatus together with others from the nuclei emboliformis and globosus pass forwards in the brachium con-

junctivum. These fibres cross the middle line and, after giving off a descending tract destined for the reticular formation of the pons and medulla oblongata, come into close association with the cells of the nucleus ruber (fig. 6). The orally-situated, small-celled component of this nucleus (nucleus ruber parvicellulatus) receives fibres from the dentate nucleus, while the aboral, large-celled component (nucleus magnocellulatus) receives fibres from the emboliform and globose

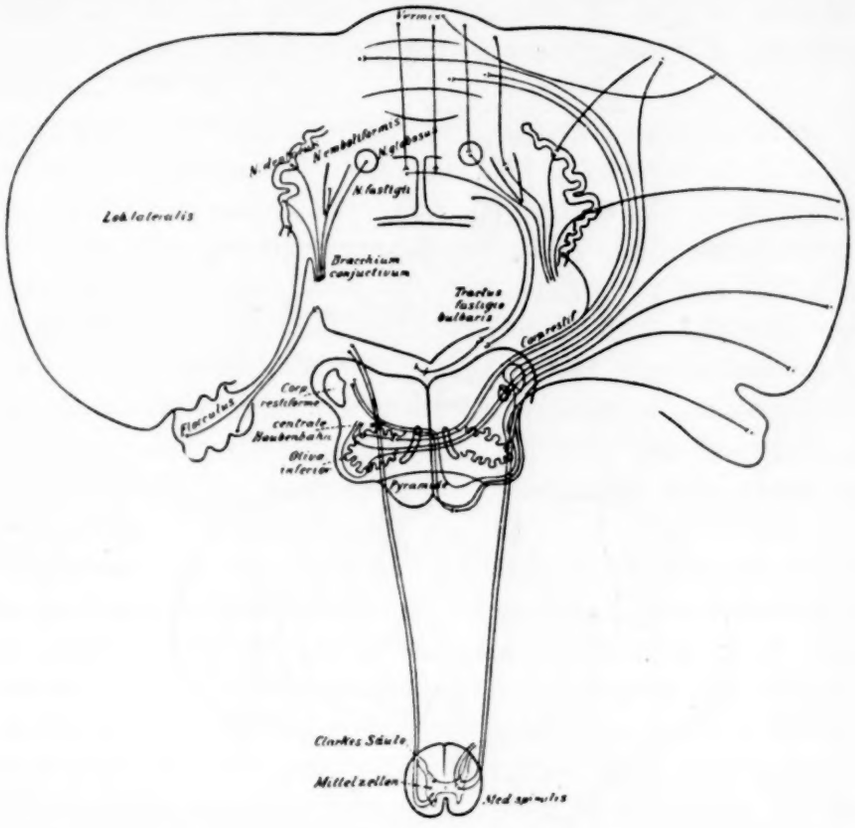

Fig. 5. Schematic representation of cerebellar pathways, spinal, bulbar and intracerebellar. (Marburg.)

nuclei (Hatschek, 1907; Marburg, 1923). The nucleus parvicellulatus is strongly developed in man, though but slightly so in lower mammals; from it originates the rubroreticular tract, which passes to the reticular formation of the pons; this tract shows a good development in man. From the reticular formation of the pons a tract passes downwards to the cord (reticulospinal tract). The nucleus magnocellulatus is rudimentary in man but well developed in the lower mammals. The rubrospinal tract, which originates in this nucleus, is correspondingly small in man but large in the lower mammals. This tract, after crossing

the middle line at the decussation of Forel, extends downwards forming collaterals around the ventral motor cells of the spinal cord. Extending in the oral direction from the nucleus ruber fibres of the brachium conjunctivum pass directly, or after relay in this nucleus, to reach the thalamus and cerebral cortex (Marburg) (fig. 6).

The nucleus fastigii gives origin to a number of tracts which leave the cerebellum by the three peduncles and end in the reticular forma-

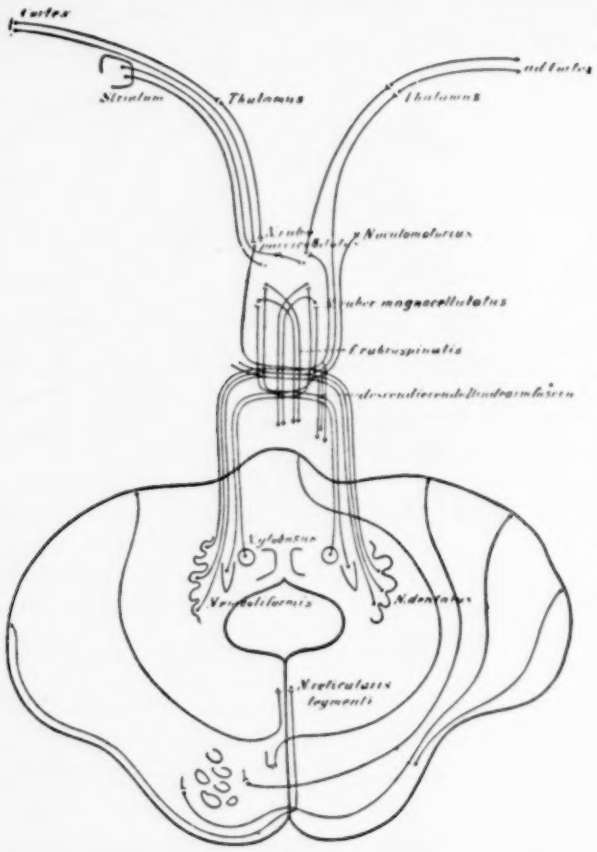

Fig. 6. Schematic representation of cerebellar pathways in superior and middle peduncles. (Marburg.)

tion of the pons and medulla oblongata, those ending in the latter situation being termed the fastigiobulbar tracts; some of these fibres are distributed to the nucleus of Deiters.

Various descending pathways from these pontobulbar centres impinge finally on the spinal motor neurones, which, in response to the influences thus conveyed to them, discharge the impulses concerned in the production of postural tone. The intricate cerebellar reflex arcs, the essential features of which have just been sketched, form the structural

basis for the complicated neural activities by which postural tone is maintained and regulated.

CEREBELLAR FUNCTIONS. The experimental methods used in the solution of cerebellar problems are of two kinds, namely, extirpation and stimulation. Extirpations have been performed in various classes of animals, the cerebellum being removed in whole or in part; while the purpose of complete removal was to throw light on the nature of cerebellar function in general, the purpose of limited excisions was usually to secure evidence in support of one or other of the theories of localisation.

EXTIRPATION OF THE CEREBELLUM IN LOWER VERTEBRATES. *Fishes.* According to Vulpian and Philippeaux (1866), removal of the cerebellum in fishes (carp and tench) is without noticeable effect; the movements, however, become greatly disorganised when the cerebellar peduncles are incised. Loeb and Steiner (Sherrington, 1900) failed to note any disturbance of locomotion in elasmobranchs following cerebellar removal. Luys (1864), however, maintained that cerebellar ablation in the fish results in slow, uncertain movements of swimming, together with some equilibratory disturbance. Clearly, the symptoms of cerebellar deficiency are insignificant as compared with those in birds and mammals.

Amphibians. In the opinion of many experimenters ablation of the insignificant cerebellum in the frog yields no perceptible symptoms. Budge (1841), however, observed that, in consequence of removal of one lateral half, for instance the right, the animal sits with the right side of the body lowered and concave, while the digits of the left hind-foot are extended; the animal can lie on the right side but not on the left; forward movements show a deflection to the right. Goltz noted that cerebellar ablation in the frog, previously decerebrated, disturbs seriously movements of locomotion, which can be performed after decerebration alone.

Reptiles. Although the older authors (cited by Sherrington, 1900) reported but little disturbance in the lizard and tortoise in consequence of cerebellar ablation, Leblanc (1923) arrived at contrary results in a recent study of various lizards. Complete extirpation of the cerebellum yielded, according to him, such symptoms as ataxia, reeling gait, retraction of the head and rotation of the body around the long axis, phenomena very similar indeed to those observed in the decerebellate mammal. Further studies in this field would appear desirable to explain the discrepancy between Leblanc's findings and those of previous workers.

Birds. Flourens' account (1824–42) of the pigeon deprived of the cerebellum formed the basis of all subsequent studies; the decerebellate pigeon cannot jump, fly nor walk; consciousness and sensibility are unaffected. Lange (1891), who continued the investigation with refined technique, took special precautions to avoid injury to other parts of the brain and the semicircular canals. As immediate symptoms he found that the animal often sits on the ground with legs drawn up; it cannot fly nor perch. When walking is attempted tetanic extensions of the legs ensue so that falling occurs and violent general movements continue until complete exhaustion. On trying to take food the head is involuntarily forced backwards. After a few weeks symptoms are present which persist more or less permanently. The gait is then ataxic and stiff-legged, the legs and front toes being rigidly extended, so that the back toe of each foot does not touch the ground. The pigeon learns to fly fairly early; it also learns to perch and can balance itself on the finger even when moved to and fro, so that maintenance of equilibrium is good. The power to take food is acquired, although the head shows a slight intention tremor during the movements.

Symptoms very similar to those just recorded were noted by Reisinger (1916) in the decerebellate rooster. Shimazono (1912) found that, following one-sided removal of the cerebellar cortex in the pigeon, the animal while attempting to stand frequently falls backwards, owing to flexion of the ipsilateral leg.

Mammals. The complete ablation of the cerebellum in the mammal was accomplished by Luciani (1891, 1915), who succeeded in keeping alive for two years a dog thus operated upon. The doctrine of cerebellar function, which he evolved by illuminating inductions from experimental data, will always remain one of the most important chapters in the physiology of this organ, while his ideas, though since modified and extended in certain particulars, form, nevertheless, the basis of our present conceptions of the physiology of the cerebellum.

A fact of fundamental importance to be stressed by Luciani is that, while lesions of the cerebrum are followed by a period of depression, lesions of the cerebellum are followed by a period of exaltation. Furthermore, no real paralysis ensues after cerebellar removal.

In the symptomatology of the animal, from which the cerebellum has been completely removed, Luciani differentiates three periods: The first is his period of "functional exaltation" or "dynamic phenomena." A dog after this operation shows agitation, opisthotonus of

the back with retraction of the head; tonic extension and abduction of both forelegs and alternating clonic movements of the hindlegs; a tendency to stagger and fall backwards; and usually a horizontal nystagmus. Without following Luciani in his efforts to interpret the meaning of these dynamic symptoms, we may state here briefly that the correct explanation is to be found in the circumstance that some regions of the cerebellar cortex discharge an influence which is inhibitory to the tone of the muscles of the animal: this influence, which was first demonstrated by Sherrington (1897–8), will receive further consideration in a later section of this article. It is in the abolition of this inhibitory action, the consequence of cerebellar ablation, that the explanation is to be sought for the symptoms of exaltation or overaction, which may, therefore, be regarded in the light of "release phenomena" (Hughlings Jackson, 1884; Head, 1921). These symptoms endure for eight or ten days, the opisthotonus being the last to disappear.

The "deficiency phenomena" of Luciani's second stage now develop, the animal showing essentially a *weakness* of all its muscles and especially those of the hindlimbs. Analysing with great shrewdness the symptoms of the second stage, Luciani recognised three primary elements:

1. Asthenia or weak motor power evidenced particularly by the muscles of the hindlimbs, which are unable to support the body.

2. Atonia (or rather *hypotonia*) or diminution of the normal tonus of the muscles; this symptom he compared to the loss of tone in the muscles of the hindleg of the frog after division of the dorsal roots in Brondgeest's experiment (1860); the comparison is apt, suggesting as it does the origin through cerebellar reflex paths of postural tonus. Atonia is, according to Luciani, the explanation of the clinical symptom *dysmetria*, in which, for instance, the movements of the fingers in seizing a pencil or other object are overestimated; this is explained by diminished tonus of antagonists as compared with protagonists.

3. Astasia, a term which includes the "intention tremors" occurring with willed movements or postures and the *titubation* or reeling.

Occurring with the deficiency symptoms just enumerated are the "compensatory phenomena;" these are seen in the more or less successful attempts to stand and walk. They are dependent, in cases of total cerebellar ablation, on functional compensation by the cerebral cortical motor centres, a fact which may be proved by removing these centres, whereupon all power to stand and walk, that has been regained, is immediately lost.

The decerebellate dog, for a long time unable to stand, first begins to do so as a result of the compensatory cerebral processes just mentioned and ultimately begins to raise itself first on the forelegs and then on the hindlegs. At this stage it can swim quite normally, demonstrating clearly that neither the "sense of equilibrium" nor motor "coördination" is lost. Standing having been relearned, it then acquires the ability to walk, at first falling frequently to one side or the other, owing to atonia of the legs, particularly the hindlegs, then eventually walking without falling at all and exhibiting the peculiarities of the "hen's gait." Of this morbid progression atonia is the cause, the diminished muscular tonus causing imperfect balance in opposed muscle groups. Functional compensation consists largely in abduction of the legs which, by enlarging the base of support, prevents the animal from falling; it is effected, as explained above, by the cerebral cortical areas. The functional compensatory activities form, in association with the residue of deficiency phenomena, the picture of *cerebellar ataxia*.

The condition of Luciani's dog, which lived for two years after cerebellar ablation was practically complete, showed progressive amelioration for the first year. There remained, however, a permanent disability revealing itself in asthenia of the hindlegs and marked intention tremors, which involved most of the muscles.

That each lateral half of the cerebellum exercises a predominant influence over the corresponding half of the body has long been known and is generally accepted; it is true that certain qualifications inherent in the theories of localisation have been introduced in recent years but it is probable that the fundamental principle is not materially altered. With this principle in mind, Luciani extirpated one lateral half of the cerebellum in the dog, and, by comparing the two sides of the animal, was enabled to interpret and analyse, even better than in the totally decerebellate animal, many of the symptoms of cerebellar ablation.

In the dog deprived of half the cerebellum the symptoms shown by the operated side are of the same order as those shown by the whole animal after total cerebellar removal. The "release" or "exaltation" phenomena include pleurothotonus or curvature of the body with the concavity on the operated side, a symptom which corresponds to opisthotonus in the completely decerebellate condition; tonic extension of the forelimb of the maimed side with clonic contractions of the three

other limbs; ocular deviations with slight nystagmus on the operated side; rotation around the long axis of the body, a classical symptom of the same order as backward falling in the animal completely decerebellate.

The deficiency symptoms reveal themselves on the injured side by weakness of the limbs so that they yield under the weight of the body, making standing impossible. The animal can, however, swim efficiently and with good equilibration, although the direction of movement is curved towards the sound side. The deficiency phenomena show themselves, on analysis, to be reducible to the three elementary components referred to in the completely decerebellate condition, namely, asthenia, atonia and astasia, involving, however, only half the animal, that on the side of the ablation.

In the course of time the falling to the injured side becomes less and less frequent until eventually the power to stand and walk is recovered; the gait remains, however, permanently ataxic. In this recovery, invariably more complete than after total cerebellar removal, the remaining half of the cerebellum plays a part, in addition to the share taken by the cerebrum, as in the completely decerebellate condition. This capacity of the residuum of cerebellar tissue to function vicariously for a portion removed (the "organic compensation" of Luciani) indicates the influence which each part of the organ may exert on the whole musculature of the body and is in itself opposed to the ideas of clearly defined regional control involved in current theories of localisation.

That consciousness and sensation are undisturbed by cerebellar removal is generally accepted. Luciani examined with particular care cutaneous sensation and muscle sense in the semi-decerebellate dog, in which recovery had attained its maximum and in which some degree of ataxia remained permanently. Light touch yielded responses equally brisk on both sides; also abnormal positions of the limbs were corrected with equal promptness bilaterally. His conclusion that cutaneous and muscle sensations are not deranged by cerebellar removal has been confirmed by numerous other observations both in the lower animals and in man.

The conception of the cerebellum as an organ of equilibration as propounded by Magendie, Ferrier, Thomas and recently by Ingvar, is opposed by Luciani, who supports his contention by the observation that the dog, totally decerebellate, can swim before it has recovered

the ability to stand or walk. Even admitting that the swimming is not completely normal, as contended by Dusser de Barenne (1923), who carried on similar studies, we may concur with Luciani's view that equilibration is not in itself a function of the cerebellum.

Luciani rejected the idea of the cerebellum as a mechanism for the coördination of movements like those of progression, holding that the ataxic gait could be explained by his triad of defects, asthenia, atonia and astasia. In accord with this view are the observations of Laughton (1924), who showed that coördinated movements of progression in fore- and hindlegs may be carried on by the decerebrate mammal deprived of its cerebellum. These results, taken with the ability to swim retained by the decerebellate dog prove that the centres of prime importance for complex muscular coördinations are not located in the cerebellum.

Ewald's doctrine of labyrinthine tonus (1892) discharged reflexly on the muscles was developed in important particulars by Luciani. Impressed with the similarity in regard to hypotonia of the decerebellate animal and the animal deprived of the labyrinths, he maintained that within the cerebellum are contained some, at least, of the reflex arcs (Stefani, 1903), which are engaged in the elaboration of this tonic influence. Although it has been shown by Magnus and de Kleyn (1920, 1924) that the cerebellum is not essential for a number of labyrinthine reflexes we must, nevertheless, recognise the probability of the participation of the cerebellum, to some degree, as an intermediary in the liberation of labyrinthine tonus. Its relationship to the medullary and spinal centres may be comparable to that occupied by the cerebral cortex, the cerebellum offering intricate reflex arcs for the more delicate grading of tonic discharges.

Luciani's doctrine of cerebellar functions may be summarised as follows: The cerebellum exerts on the body muscles a coadjutant or reinforcing influence, which is *tonic*, i.e., maintains the tonus of the muscles; *sthenic*, i.e., increases the energy of muscular movements; and *static*, i.e., maintains the steadiness of muscular contractions. That these activities are aroused reflexly in the cerebellum by proprioceptive impulses from the labyrinths as well as from the muscles, the conception held at the present time, was clearly implied in Luciani's writings.

Dusser de Barenne (1923) has recently made extensive studies of the symptomatology of cats and dogs deprived of the whole or half the cerebellum; his experiments were carefully controlled anatomically.

Hypotonia as a cerebellar defect he denies, being unable to detect it in the limbs of the maimed side, when the head of a semidecerebellate animal was held straight so as to exclude the influence of the "neck reflexes" of Magnus and de Kleyn (Magnus, 1924). An opportunity was afforded to examine the labyrinthine reflexes (Magnus, 1924) in the totally decerebellate dog and it was found, in harmony with previous observations on decerebellate rabbits and cats, that these reflexes are present. In spite of Barenne's failure to demonstrate hypotonia under the conditions of examination we may well feel scepticism regarding his conclusion that it is absent, particularly in view of Holmes' unqualified assertions as to its presence in man after cerebellar lesions (vide infra).

Using the method of cerebellar extirpation, Rossi (1912a) obtained evidence of a "reinforcing action" of the cerebellum on the cerebrum: he found that ablation of one lateral cerebellar half in the dog caused in the opposite cerebral motor area first a diminution and then an augmentation of excitability. Thus each lateral half of the cerebellum "reinforces" the motor discharges from the contralateral cerebral motor cortex.

The investigation by Gordon Holmes (1917, 1922) of cases of cerebellar injury resulting from wounds received during the War must rank together with the classical researches of Luciani as among the most significant contributions ever made to the physiology of the cerebellum. Through the voluntary coöperation of the patients it was possible to investigate many problems, the solution of which by experimental methods is difficult or impossible; thus many obscure matters were explained, while new and significant phenomena, important alike to clinical neurology as to theoretical physiology, were observed and accurately analysed. Holmes related his work to that of Luciani, Babinski (1899) and André-Thomas (1911), although qualifying and extending the conclusions of these authors in important particulars.

The failure to observe any of the immediate "release (dynamic) phenomena," so prominent in the animal after cerebellar ablations, was remarkable and may, perhaps, be attributed to the type of injury or the inevitable delay before examination; Holmes' studies, therefore, deal with the second and third stages of Luciani, namely with the symptoms of cerebellar deficiency and of organic and functional compensation.

Reviewing the symptomatology described by Holmes we note that he always found atonia or hypotonia affecting the muscles of the limbs and, though to a less extent, those of the trunk on the side of the cerebellar lesion. It is readily revealed by flabbiness to palpation and by the greater effect of gravity in bending the wrist of the affected arm as compared with the normal wrist. In the leg it is shown by the pendular character of the knee-jerk (fig. 7), though the incidence of hypotonia is less severe in the leg than in the arm.

Asthenia or weakness of the muscles of the injured side is illustrated by the lessened force shown by the dynamometer as well as by the observations of the patients themselves on the weakness and uselessness of the affected limb.

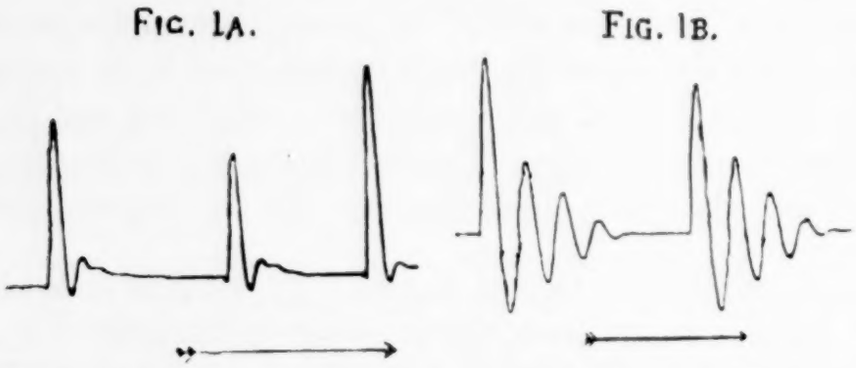

Fig. 7. Tracings of normal knee-jerk, *1A*, and of that on side of cerebellar lesion, *1B:* hypotonia of affected leg. (Holmes.)

Astasia, though not an invariable symptom, is shown in the muscular tremors, the contractions being of a discontinuous, clonic nature, consequent on the imperfect sustaining of the contraction process (fig. 8).

A conspicuous symptom investigated by Holmes was the delay in commencing and terminating muscular contractions, abnormalities clearly revealed by graphic recording; to this delayed relaxation he attributes dysmetria shown in the exaggerated scope of movements, which often overshoot the mark. Babinski's statement that this symptom depends on deficient normal "braking" action of the cerebellum is an interesting suggestion when taken in relation to the inhibitory action of parts of the cerebellar cortex (vide infra).

Decomposition of movement is frequently met with: for instance, in placing the heel of the affected leg on the opposite knee the heel is

first dragged along the bed and then raised to bring it to the knee: as Holmes expresses it, the act is performed "by numbers," as in military drill.

Asynergia, depending on lack of coöperation among associated groups of muscles, is also common: for example, a patient while standing, on throwing his head back, often falls, as a result of not flexing his knees, whereby his balance would be preserved.

Babinski's symptom, *adiadochokinesis*, the inability rapidly to perform alternate movements, is frequently encountered: thus, pronation and supination are performed in a disordered way by the affected arm (fig. 9).

Fig. 8

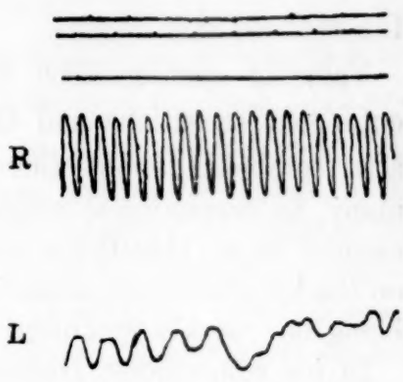

Fig. 9

Fig. 8. Tracings of flexion of right, *R*, and of left, *L*, forearms against a spring in a case of right-sided cerebellar lesion: astasia of affected arm. (Holmes.)

Fig. 9. Tracings of rapid alternate supination and pronation of the right, *R*, and left, *L*, arms in a case of injury of the left side of the cerebellum: adiadochokinesis in left arm. (Holmes.)

Results opposed to the existence of Bárány's hypothetical "tonus centres," (vide infra) are evident in the deviation of the affected arm, which is usually in an outward direction, either in performing the vertical "pointing test" or as a "spontaneous" phenomenon, both arms being extended in front of the body. The eyes at rest are generally turned to the sound side, a symptom related, no doubt, to Ferrier's results on stimulation.

Nystagmus, the relationship of which to cerebellar injuries has been questioned by several physiologists, is an almost invariable symptom:

it becomes pronounced on directing the eyes to the injured side, the quick movements being towards the fixation point, the slow movements towards the resting position. Differing essentially from the nystagmus of labyrinthine injury, Holmes regards it as a purely cerebellar defect symptom, consequent on disordered postural tone in the eye muscles.

Disorders of phonation and articulation are revealed in the mode of speech, which is jerky and irregular, peculiarities which may be attributed to muscular derangements like those in the rest of the body. The gait in unilateral injury is characteristically ataxic, showing, on the affected side, either the exaggerated movements of the step or the dragging of the foot along the ground, effects familiar in the animal and ascribed by Luciani to atonia; deviation of the patient towards the maimed side results.

Valuable corroboration is afforded for Luciani's assertion that cutaneous sensations and the sense of position are preserved intact after cerebellar lesions; there is, to be sure, a tendency on the part of many, to overestimate weights placed in the affected hand but closer enquiry shows that the error depends not on a true sensory defect but on the hypotonic and asthenic condition of the limb, the proprioceptors being but weakly stimulated.

In his conclusions, Holmes is in agreement with Rossi (1921a) in emphasising the greater importance of atonia (hypotonia) as a symptom of cerebellar defect as compared with asthenia and astasia. He believes that the cerebellum influences postural tone in two ways, first by a continuous discharge for the maintenance of attitudes and secondly by a discontinuous discharge for the regulation of the tone that accompanies muscular relaxations and contractions.

Holmes is not disposed to favour localisation in the cerebellar cortex. He writes: "I have invariably found that the symptoms produced by a small lesion were never limited to one limb or a segment of a limb; that if any abnormality could be detected in the arm the leg was affected too, and that there was very frequently in addition some disturbance in the functions of the cranial nerves." He concedes, however, a "prevalence of representation" (Rossi) for the arm anteriorly and for the leg posteriorly in the lobulus ansiformis. With respect to the location by Bolk and others of centres for the head and larynx in the lobus anterior (vide infra), it must be stated that Holmes' observations are greatly at variance, since he found defects in ocular

movements and disorders of speech in consequence of lesions in parts other than the lobe in question.

CEREBELLAR LOCALISATION. Some attention will now be devoted to the theories of cerebellar localisation, already casually mentioned in previous parts of this paper. Starting from the generally-recognised functional relationship existing between each lateral half of the cerebellum and the corresponding half of the body, Bolk (1906) formulated an elaborate schema of localisation based on hypotheses of a highly speculative character, into which it is impossible to enter here save in the briefest fashion. To cite a few instances of Bolk's method of reasoning we may mention that he placed the "coördination centre" for the neck muscles in the lobulus simplex, since he found this lobule relatively small in the ant-eater and mole and relatively large in the giraffe. He argued that in some cases the muscles of both sides function mainly together, as for instance the muscles of the head, neck and trunk: for each of these there must be an unpaired centre to "coördinate" the two muscular halves. These centres he placed in the median parts of the cerebellum. In other cases, as for instance the limbs, each muscular unit acts more or less independently and therefore a centre in the lateral cerebellar lobe is essential; but here again a medially-placed centre is required for synergic movements of the two sides. He placed centres for the head in the front of the cerebellum and then followed backwards, allotting centres in sequence to the corresponding muscular territories, in doing so observing the principles outlined above. His results may be briefly summarised as follows:

Lobus anterior—muscles of head, including those of the eyes, tongue, the masticatory and facial muscles and besides the muscles of the larynx and pharynx.

Lobulus simplex—muscles of the neck.

Lobulus C_2—unpaired centre for right and left extremities.

Lobuli ansiformis and paramedianus—centres for ipsilateral limbs.

Lobuli "a" and "b" (i.e., nodule and uvula)—centres for trunk.

Formatio vermicularis (lobus flocculi)—centres for trunk and tail (ideas very vague).

This localisation schema, embodying also the ideas of Rynberk, next to be described, is shown in relation to Bolk's nomenclature in figure 10; it will be remembered that since Bolk, unlike Ingvar, does not distinguish a lobus medius his lobulus medianus posterior represents the entire median cerebellar part behind the lobulus simplex instead of

simply that behind the sulcus praepyramidalis; otherwise, there is much similarity between the two nomenclatures.

It is impossible to read Bolk without feeling serious scepticism as to the validity of his theory, particularly since his basic assumption of "coördination centres" is opposed to Luciani's well-established theory. Even admitting these centres to be "regulative" simply we must doubt whether the cerebellar cortex can be charted out as in Bolk's schema, because of the capacity, already cited, of one part of the cerebellum to compensate for other parts destroyed or removed.

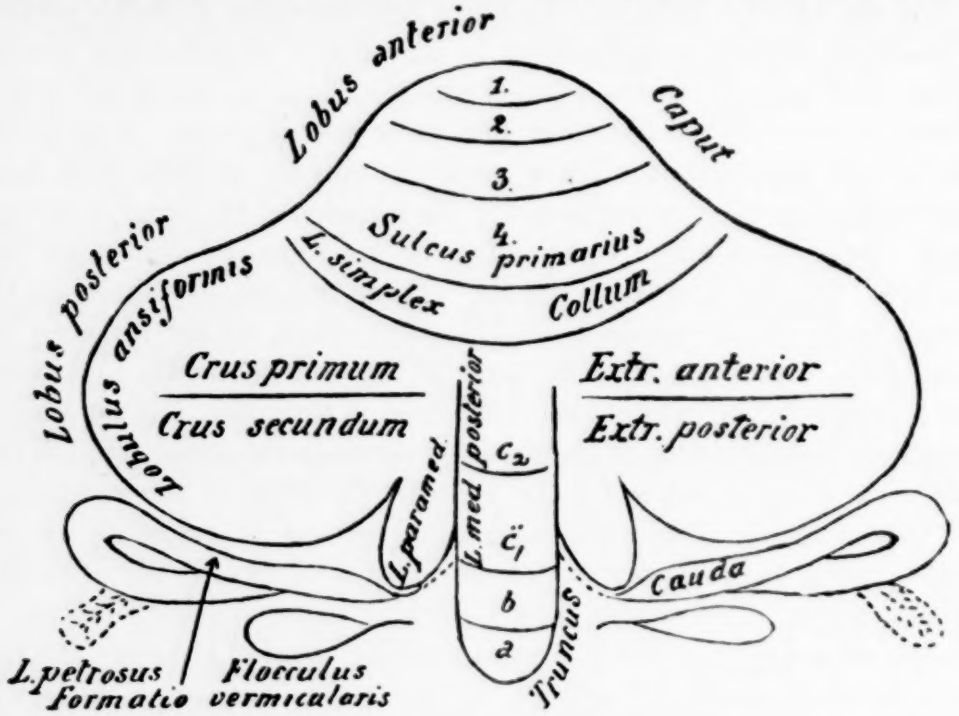

Fig. 10. Schematic representation of the Bolk-Rynberk theory of localisation (right side) in relation to Bolk's nomenclature (left side). (Luciani.)

Surely this would be impossible with the strictly regional representation assumed by Bolk. To these objections others of a still more serious character will be considered in succeeding portions of this article. It is true that several workers claim to have confirmed Bolk's theory but an impartial examination of their results reveals not only differences from Bolk but great discrepancies between the separate investigations. The case for localisation has been presented recently by Rynberk (1925); here the main lines only will be followed.

Rynberk (1908) was the first to undertake the systematic experi-

mental control of the theories of Bolk and his results, obtained in the dog, will now be related:

Lobulus simplex—partial destruction of this lobule yields a "head astasia" or "head nystagmus," the head oscillating laterally as if signifying "no." Hence the "centres" for the neck are in this lobule.

Crus primum lobuli ansiformis—lesions here cause in the ipsilateral foreleg the "military salute," the paw being raised to the ear in response to a pinch or noise. Later the "hen's gait" develops in the affected limb. The centres for the ipsilateral foreleg are, therefore, in this lobule.

Crus secundum lobuli ansiformis—destruction of the medial knee of this lobule results in slight, though definite weakness in the ipsilateral hindleg. Thus this lobule encloses the centres for the ipsilateral hindleg.

Lobulus paramedianus—extirpation of this lobule yields longitudinal rolling and pleurothotonus, which is believed to show that here are the centres for the ipsilateral trunk muscles.

Lobulus C_2 (tuber or lobul. "S")—extirpation of this lobule by itself never produces a noteworthy disturbance, although when combined with removal of the crus I it intensifies the effect consequent on this latter lesion. This last result, when considered by Rynberk in connection with similar extirpations in the sheep's brain, in which C_2 is large, is regarded by him as confirming the view that this lobule contains the unpaired centre for the extremities of both sides.

While Rynberk considers that his experiments supply confirmation of Bolk's schema close examination reveals a lack of complete agreement in certain details: for example, Bolk includes the lobulus paramedianus together with the lobulus ansiformis as embracing the centres for the ipsilateral limbs, whereas Rynberk regards the former lobule as containing the centres of the ipsilateral trunk muscles.

Among the experimenters who performed cerebellar ablations from the standpoint of localisation we may mention Rothmann (1914), André-Thomas and Durupt (1914), Grey (1916), Luna (1918), Rossi (1921b), Simonelli (1921) and Ten Cate (1925). Most of these writers agree with Rynberk as to the symptoms following removal of the lobulus simplex, of the crus I and the crus II; in other respects, however, there is great diversity of opinion. For example, Rothmann places in the lobus anterior, besides representation for the muscles of the head, as well representation for those of the neck, trunk and limbs, since

retraction of the head, lordosis of the vertebral column and extension of the forelegs ensue on removal of this lobe.

André-Thomas and Durupt, who consider the foreleg centre to be in the crus I, subdivide it, locating a centre for abduction in the medial half and a centre for adduction in the lateral half of this lobule. The adduction of the foreleg consequent on the destruction of the abduction centre in the medial part of the crus I is shown in figure 11, the site of the lesion itself being shown in figure 12. They express the old and doubtful view that the "vermis" is for equilibrium. Rossi and Simonelli showed that a very superficial lesion of the cortex of the crus I caused

Fig. 11

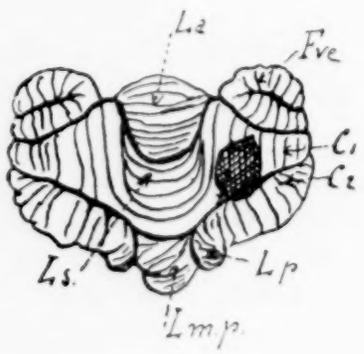

Fig. 12

Fig. 11. Adduction of right foreleg in consequence of lesion shown in next figure; a couple of days after operation. (André-Thomas et Durupt.)

Fig. 12. Lesion in medial half of crus I. Nomenclature according to Bolk. (André-Thomas et Durupt.)

only a slight flexion of the foreleg, a deeper lesion, though still affecting only the cortex, yields the "military salute" and "hen's gait." Ten Cate considers that the paraflocculus is in functional relationship with the ipsilateral extremities, a conclusion entirely at variance with the function attributed to this lobule by Bolk; nor is there compatibility with the theory of Rynberk which implies the complete representation of the fore- and hindleg in the crus I and crus II respectively.

Bárány (1912, 1913, 1922), who is an adherent of Rynberk's theory of localisation, maintains the existence of four tonus centres in each hemisphere for the control of tonic movements of the respective limb

in corresponding directions. There is a centre for movement outwards, a centre for movement inwards, a centre for movement downwards and a centre for movement upwards. The tonic influence of these centres is envisaged by Bárány in accordance with Luciani's principles of cerebellar action. The location of the centres so far determined by Bárány is as follows.

The centre for downward tonus of the upper extremity is in the medial, posterior end of the superior and inferior semilunar lobules. The centre for outward tonus of the arm is at the outer border of the hemisphere in the region of the superior and inferior semilunar lobules. The centre for inward tonus of the wrist is in the most anterior part of the biventral lobule. Outward and backward from this is the centre for inward tonus of the arm. Still farther outward and backward is the centre for inward tonus of the hip. These centres, when functioning together normally, maintain the limb in equilibrium. When one of the four centres for a limb is paralysed the opposite centre acts without control and the limb shows a corresponding deviation. Bárány cites the following evidence in support of the existence of these centres: Sudden damage of the region occupied by a centre causes spontaneous "pass-pointing" (Vorbeizeigen) or deviation in performing his "pointing test." (See Tilney and Riley, 1921.) For instance paralysis of the inward tonus centre in the right hemisphere for the right arm causes in this extremity pass-pointing to the right, the outward tonus centre being unopposed.

Bárány was able to show experimentally that cooling a given centre abolishes temporarily its tonus action. The cooling he accomplished by a modification of the method of Trendelenburg for cooling the centres of the cerebral cortex. In a number of cases of Bárány, in consequence of operative procedures, the bone over the cerebellum was absent and the hemisphere was covered only by the dura and a thin layer of skin. Bárány applied ethyl chloride, which served to cool, through the skin and dura, the cerebellar cortex at the tonus centre in question. As a consequence, the respective limb showed spontaneous pass-pointing resulting from the unopposed action of the antagonistic centre.

Bárány had shown that pass-pointing can be induced in the normal individual, together with nystagmus, by his caloric test; the pass-pointing takes place in the direction of the slow movement of the nystagmus; he considers the cerebellar cortex to be the intermediary in the

tonus concerned in pass-pointing. If now the cerebellar centre, which Bárány assumes to be involved in this pass-pointing, is cooled the pass-pointing ceases. This observation confirms, for Bárány, his assumption that the pass-pointing was produced by a tonus influence discharged from the cortical centre.

While Bárány states that his results on localisation are in complete agreement with those of Bolk, Rynberk and Rothmann, close scrutiny of his observations reveals discrepancies, since, unlike these writers, he locates certain arm centres posterior to the centres for the leg. Further evidence opposed to Bárány's centres is found in Holmes' cases (vide supra) in which, though many separate regions of the cerebellum were damaged, pass-pointing is almost always outwards; hence there cannot be an inward tonus centre. Finally, the proof by Magnus and de Kleyn that the cerebellum is not essential for their labyrinthine reflexes removes much of the support for Bárány's postulate that the cerebellar cortex is an intermediary in the tonus of pass-pointing evoked from the ear.

Opinions strongly opposed to Rynberk's theory were expressed by Troell and Hesser (1922), practically the only point of agreement being in regard to the functions of the crus I, the relationship of which to the foreleg they admit. The crus II, they think, is not only related to the hindleg but to the foreleg as well. The lobus anterior is not related to the head but to the trunk and limbs. Other divergences even more striking are mentioned so that, as a whole, the paper is a strong protest against Rynberk's theory.

Rossi (1913) was not successful in showing any change in the excitability of the cerebral cortex in consequence of removal of the crus I and crus II, although he noted alterations when half the cerebellum was removed (vide supra). These experiments, therefore, argue against separate centres.

The results outlined above, though highly divergent, show some agreement in regard to the symptoms induced in the neck and foreleg by lesions of the lobulus simplex and crus I respectively. The location in these lobules of "centres" for the neck and foreleg does not, however, appear justifiable, for the reason that disturbances, though admittedly of a different kind, may be provoked in the neck and arm by damage of lobules other than those specified. In regard to the lobus anterior the location there by Bolk of centres for the head is certainly incorrect since it receives spinocerebellar fibres from all segments of the cord

(Ingvar); furthermore, its removal evokes symptoms in parts other than the head, as for instance the neck, back and forelegs. Grave doubts must exist then as to the validity of Bolk's and Rynberk's theory. Against it is the observation, familiar to everyone who has performed cerebellar extirpations, that a small lesion yields wide-spread disturbances; the utmost we may admit then is a "prevalence of representation" (Rossi) in certain locations, that is, that the arm is represented more anteriorly and the leg more posteriorly in the lobulus ansiformis, though the centres for both extremities are intermingled. Indeed many facts favour Luciani's view that the cerebellum functions as a whole, since, although the control of each cerebellar half is predominantly over the same side of the body, each half can compensate to a considerable extent for the deficiency of the other half.

The theory of localisation recently advanced by Ingvar (1918, 1923) is founded on extirpations carried out in conformity with his subdivisions of the cerebellum. He holds that bodily equilibrium is maintained as a result of reflex activities in different parts of the cerebellum, the muscles being so controlled that falling in different directions is opposed. In this process the lobus anterior dominates the muscles which prevent falling forwards; the lobus posterior, or rather the median part of it, the lobulus posterior medianus, dominates the muscles which prevent falling backwards. In the lobulus medius medianus (of lobus medius), in which, it may be recalled, no spinocerebellar fibres end, no influences are exerted on equilibrium. For the opposing of the force of gravity laterally Ingvar calls in the action of the centres in the crus I and crus II, which are supposed to regulate the tonus in the fore- and hindleg respectively; he thus introduces principles of Rynberk's theory into his doctrine. Although it is doubtful whether the conception of the cerebellum as an organ of equilibrium can be logically sustained in the face of Luciani's criticisms (vide supra) nevertheless Ingvar's theory, by its originality and suggestiveness, forms an important contribution to cerebellar physiology.

Reference must now be made to one of the most curious and remarkable discoveries ever made in the realm of cerebellar physiology. Magnus (1924) and his associates in the University of Utrecht have made, in recent years, extensive studies on the reflexes of posture, by which the various attitudes of the body are assumed in response to the changing needs of the organism. These reflexes include the "tonic labyrinthine reflexes," evoked through the agency of the otoliths act-

ing on the maculae of the utricle and saccule; the "tonic neck reflexes" elicited from the proprioceptors of the neck; and the various "righting reflexes." All these reflexes have as their motor expression, alterations in the postural tone of various body muscles. They are indeed just the kind of responses that we would naturally be inclined to attribute to cerebellar action. Nevertheless, Magnus and his collaborators have found that all these postural reflexes persist apparently unaltered after complete removal of the cerebellum.

While then the cerebellum is not essential to these reflexes we must still attribute to it an important rôle in relation to muscular tone. The evidence here is in part derived from the loss of tone following ablations, in part also from changes in tone produced by stimulation of the cerebellum itself, changes which may be of the nature of augmentation or inhibition (vide infra). Finally, the arrangement of the cerebellopetal and cerebellofugal tracts suggests the activity of the cerebellum to be such as we have sketched. Possibly, the rôle of the cerebellum in regard to tonic reflexes may be likened to that of the cerebral cortex, which, though not essential to many complex activities executable by subcortical centres alone, still adds elements of delicate modification and control to these activities of the lower centres.

ELECTRICAL AND CHEMICAL STIMULATION OF THE CEREBELLUM: THE EXCITABILITY OF THE CEREBELLAR CORTEX. The effects of faradisation of the cerebellar cortex have been studied by a large number of experimenters, especially by Ferrier (1886), Mendelssohn (1898), Wersiloff (1899), Prus (1903), Negro and Rosaenda (1907) and Lourié (1907). This work, having been already reviewed by Rynberk (1908) and by André-Thomas (1911), may be passed over here with but brief mention. While the ocular deviations, nystagmus and tonus changes observed by Ferrier impress one as due to genuine cerebellar stimulation this statement cannot be made regarding much of the other work: here many of the results are obviously fallacious and depend on spread of current (those used were generally very strong) beyond the cerebellum to various tracts and nuclei, more particularly to the nucleus and rootlets of the accessorius nerve.

The serious discrepancies between the results of stimulation observed by different workers together with the great intensities of current which they frequently employed induced Horsley and Clarke (1908) to deny the excitability of the cerebellar cortex and to ascribe the effects occasionally yielded by cortical stimulation to current diffusion to the intracerebellar nuclei.

The same view was expressed by Beck and Bikeles (1912, 1914a) in consequence of their failure to observe effects from the application of strychnine and phenol to the cortex, procedures previously adopted by Magnini (1910). Beck and Bikeles are guilty of inconsistency, however, since, after denying the excitability of the cortex in one paper, they assert subsequently (1914b) that cooling does not depress the *excitability* of the cortex. Their failure to mention any definite reactions in the latter instance makes it appear probable that, whatever responses may have been induced, they owed their origin to action of the current elsewhere than on the cortex (trigeminus, accessorius or nucleus gracilis or cuneatus). Such an interpretation would afford a plausible explanation of the lack of change on cooling. Similar criticisms apply to the conclusions of Bikeles and Zbyszewski (1914) that cocaine does not lower the excitability of the cortex.

The negative results of Beck and Bikeles with strychnine are controverted by those of Shimazono (1912), who observed an ipsilateral increase of tonus after application of the drug to the cerebellar cortex in the pigeon. I myself (Miller, 1920) have observed an augmented tonus in consequence of applying strychnine to the cortex cerebelli of the cat, care being taken to preclude diffusion of the drug to the medullary centres. Thus, while admitting certain inherent limitations in the use of strychnine, the evidence thus far available is clearly in favour of the view that the cerebellar cortex may be excited by this drug.

Reverting now to the subject of faradisation of the cortex the idea that its neurones are susceptible to this type of stimulation appears natural and logical and its proof demands only the selection of a suitable test reaction, which might be evoked by currents of low intensity, the action of which would extend but a slight distance below the surface.

A reaction fulfilling these conditions was described by Sherrington (1897–8): when the rostral surface of the cerebellum is faradised there ensues an immediate inhibition of the "decerebrate rigidity" previously evoked by ablation of the cerebral hemispheres. This inhibitory response was employed by Banting and myself (1922 a,b) as a means of deciding the question whether the cerebellar cortex is excitable. The study of a preparation in decerebrate rigidity has the obvious advantage that, the cerebellum being a "tonus apparatus," any alterations of tonus evoked by its stimulation will be immediately manifest against the "back-ground" of the rigidity.

In our work ablation of the cerebral hemispheres was performed with Sherrington's decerebrator (1914–15), a preparation (cat) being thus obtained in which the front surface of the cerebellum was readily available for stimulation. Bipolar stimulation was used in order that the current might, as far as possible, be confined to the cortex. We came to the conclusion that the inhibition of decerebrate rigidity elicitable from the cerebellum is in reality dependent on a state of excitation in the cortical neurones themselves. The evidence furnished by our observations in support of this deduction may be shortly summarised.

In the first place the reaction (inhibition of tonus) can be elicited with a very weak current, weaker in fact than is necessary to excite the cerebral motor cortex, the excitability of which is generally recognised. Secondly, it is essential for the success of the response that the cerebellar cortex be in a healthy, vascular state, the development of anaemia immediately abolishing the effect; herein lies a close parallelism with the cerebral cortex. Furthermore, we found that the response could be annulled by the superficial application of cocaine, the depressing action of which must be mainly on the cortical neurones. These data lead logically to the conclusion that the inhibition of tonus is effected in consequence of a state of excitation in the cortical neurones or, in other words, that the cerebellar cortex may be excited by the faradic current. This action of the cerebellum probably corresponds to a normal functional activity: hence there is a rational basis for Babinski's "braking" or inhibiting action.

Mention was already made of a "reinforcing action" of the cerebellum on the cerebrum; in further proof of this action we have the experiments of Rossi (1912b), who showed that faradisation of one lateral half of the cerebellum raised the excitability of the cerebral motor cortex of the opposite side. Incidentally these experiments add support to the view that the cerebellar cortex is excitable.

Clinical symptoms of cerebellar disease. It is beyond the scope of this article to make more than brief reference to the clinical symptoms of cerebellar disease. For complete accounts the admirable writings of Babinski, André-Thomas, Tilney and Riley, Purves-Stewart (1924) and others are available.

We may allude first briefly to symptoms of faulty development of the cerebellum. In a case of almost total absence of the cerebellum, the patient, a girl who lived to 13 years of age, was able to walk, though

she fell frequently owing to great weakness of the legs; speech was difficult and hesitating. When only part of the cerebellum is wanting symptoms may be slight or altogether absent: in a case of deficiency of almost the whole right hemisphere symptoms like weakness and nystagmus were lacking, only a slight head-turning to the left with oscillations being present in youth (André-Thomas, 1911). These cases illustrate a fact already emphasised, namely, the remarkable degree to which compensation for cerebellar defects by the remaining portions of the cerebellum is possible.

Among the cardinal symptoms of cerebellar disease we may mention the following, which may be passed over briefly, since most of them were discussed above in the review of the work of Holmes:

Hypotonia, perhaps the elementary component of most cerebellar defects.

Difficulty in standing, a disability which, unlike that in tabes, is not intensified by closing the eyes (Romberg's sign negative).

An ataxic gait with reeling and compensatory movements to enlarge the base of support.

Adiadochokinesis, the inability rapidly to perform alternate movements.

Dysmetria or misjudgment of movements.

Intention tremors (like those of disseminated sclerosis) more prominent in later stages and illustrated in the handwriting of the patient.

Decomposition of movement or splitting of movements into separate acts.

Asynergia (Babinski) or defective capacity for associated and allied movements.

Catalepsy (Babinski) or a tendency to maintain an attitude longer than a normal subject.

Nystagmus and disorders of speech.

The interpretation of many of these phenomena is given in the work of Holmes outlined above and reference may be made to it and to the various treatises on clinical neurology for more complete discussion.

DISCUSSION AND CONCLUSIONS

It is time now to review the experimental and clinical data presented in foregoing sections of this paper and to formulate, in accordance therewith, a doctrine of cerebellar function. The attainment of our

goal is fraught with considerable difficulties since the data are often hard to interpret, with the result that the views of various authors differ widely. The doctrine of cerebellar function now to be propounded appears to me the most plausible; perhaps a somewhat dogmatic exposition of it is justifiable in the interests of clarity.

Proprioceptive impulses arrive at the cerebellum from the labyrinths and from the muscles, mainly, though possibly also from the tendons and joints, and arouse, in the cerebellar reflex centres, efferent discharges which maintain and regulate the tone of the muscles of the body in correspondence with varying needs. Muscle tone we must conceive, on the basis of Sherrington's teaching, as being distributed throughout the musculature conformably with the various postures assumed by the body: in other words, muscle tone is always "postural tone."

The views of Sherrington just referred to are the outcome of his analysis of "decerebrate rigidity" (1897–8, 1910, 1915, 1924; Liddell and Sherrington, 1924), a condition of "reflex standing" which ensues on decerebration by a transection through the superior colliculi, the red nucleus being involved in the section (Rademaker, 1924). In this condition postural tone is expressed by hypertonus of the "antigravity muscles" accompanied by hypotonus of their antagonists, the due allotment of tonus being accomplished by reflex processes of coördination like those occurring in the reflexes of movement: important among these is "reciprocal innervation": the afferent impulses come from the proprioceptors (muscle-spindles?) in the muscles actually engaged in sustaining the attitude, the exciting stimulus being the mechanical stretching of the muscles caused by gravity acting on the body.

Although in decerebrate rigidity the hypertonus of the antigravity muscles and the hypotonus of their antagonists are both exaggerated in comparison with normal standing it seems legitimate to infer that the same principles are at work in both conditions: thus, in normal standing the antigravity muscles will show augmented tonus, their opponents diminished tonus, this state of postural tone being brought about by reflex coördinations similar to those operating in the decerebrate condition, although they are undoubtedly of a more complex and refined character.

Let us now return to the cerebellum. Recognising from the evidence already given that the cerebellum is a tonus mechanism in-

fluencing tonus in the way of augmentation or inhibition, we must conceive it as being concerned with postural tone, since that is the only kind which exists in the body. The function which we may assign to it here is that of regulating and refining the crude adjustments of tonus effected by the spinal and prespinal centres; for these crude tonus adjustments the cerebellum is not required, as is shown by the fact that the extreme tonus of decerebrate rigidity continues after cerebellar removal. The relationship of the cerebellum to postural tone may, it seems to me, be established by the following considerations: The hindlegs of the spinal dog can stand (Sherrington, 1915), less efficiently it is true than they can in the decerebrate dog, but nevertheless they can do so: hence the reflex arcs of the spinal cord are competent to function for the postural activity of standing. With the centres of the medulla oblongata intact as far forwards, approximately, as the nucleus of Deiters (Bazett and Penfield, 1922) standing in the hindlegs becomes more perfect, while, at the same time, standing in the forelegs is rendered possible, because with the more extensive prespinal reflex arcs here present coördination is more perfect: thus we have the standing of decerebrate rigidity. With the thalamus or the red nucleus (Rademaker) present standing is yet more perfect, through the more accurate coördination of muscle tone by the complex reflex arcs available. We may now infer that the cerebellar reflex arcs will act in a manner like that of the prespinal arcs, so that by their yet more complicated activities the tone required for posture and movements is graded and delicately regulated.

That the normal distribution of postural tone depends on the red nucleus and rubrospinal tracts was proved by Rademaker, since if either of these is injured rigidity appears; this activity of the red nucleus in the regulation of tone we must conceive to be reflex in nature, dependent upon spinal and prespinal connections: but in addition there is an influx of cerebellar impulses, themselves reflex in origin, passing from the globose and emboliform nuclei to the large-celled part of the red nucleus: these cerebellar impulses must, therefore, influence the tonus discharge of the red nucleus. Inferentially we may assume a similar control by the nucleus dentatus over the small-celled component of the red nucleus.

Thus we may form some slight conception of the way in which postural tone may be affected through the elaborate reflex activities of the cerebellum: and this cerebellar influence may be exerted either in

the direction of augmentation, as proved by cerebellar ablation, or of inhibition, as proved by faradisation of the anterior cerebellar surface.

Coming now to the labyrinths and their influence on muscle tone (Ewald), it is necessary to state that they are not essential to the production of the extreme tonus of decerebrate rigidity (Sherrington, 1897–8). Despite this observation we must still attribute to the labyrinths influences exerted on normal postural tone through cerebellar pathways even though these influences are not yet clearly defined: important considerations here are the extensiveness of the vestibular connections, taken with what we know of cerebellar function in general. Indeed, with respect to both vestibulocerebellar and spinocerebellar reflex processes I wish to reiterate a comparison made earlier between the influence of the cerebellum and that of the cerebral cortex on centres of the brain-stem and cord: thus the cerebellum offers arcs of higher levels for the elaboration of more refined reflex tonus activities.

Besides these reflex influences on tone the cerebellum probably exercises a control of a strengthening or sthenic nature over voluntary movements, there being available for this action extensive connections between the cerebral cortex and cerebellum by way of the pontine nuclei (Howell, 1924). The cerebral centres can thus play upon the cerebellum and this in turn can play upon centres in the brain-stem and cord. Furthermore, the cerebellum can "recoil" (Sherrington, 1900) through the superior peduncle, red nucleus and thalamus on the cerebral cortex and thus "reinforce" the action of the cerebral motor neurones, as was asserted by Luciani and proved experimentally by Rossi.

In these tract relationships we find the structural basis for the part of Luciani's sthenic, tonic and static functions, which relate to cerebral activities, voluntary movements being strengthened and rendered more continuous. In rupture of these pathways, on the other hand, is to be found the explanation for such voluntary disturbances as adiadochokinesis (Babinski) and the delay in beginning and terminating voluntary contraction.

With respect to functional localisation in the cerebellar cortex it must be stated that the available evidence when impartially examined does not support the existence of separate "centres." More plausible would appear to be a theory, which would attach due importance to

the sites of termination of the various categories of cerebellopetal fibres, as determined by Ingvar. As matters stand at present much may be said in favour of a rather "uniform" function of the cerebellum, though we may perhaps admit a "prevalence of representation" of muscle territories in certain parts; in any case there is an intermingling of the centres.

To summarise briefly, we have in the cerebellum a series of intricate reflex centres, which, in response to streams of proprioceptive impulses from the labyrinths and muscles, emit finely graded impulses which assist, in a delicate manner, in sustaining and "regulating" the postural tone of the muscles in conformity with the attitudes and movements of the body. To these complex activities we may add a "reinforcing" action of somewhat similar nature on voluntary movements.

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FUNCTIONS OF THE SPLEEN

(*Mysterii Plenum Organon*) GALEN

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A generation ago a critical review of the functions of the spleen might well have begun with the story that Asher attributes to du Bois Reymond. The great physiologist having asked a candidate in a state examination what he knew about the function of the spleen, the embarrassed young man stammered "I can't tell you just now, I have forgotten it." "What a pity," said his questioner, "because you were evidently the only man who knew anything about it." Today, however, we can assert without fear of valid contradiction that the spleen is the largest lymphoid structure in the body; it is intimately concerned with blood cell destruction and potentially with blood cell formation; it has a controllable reservoir system of importance in aiding the circulation in volume changes of various kinds. Other probable functions in relation to immunity, digestion, metabolism, tumor formation and so forth are still sub judice, though many positive data have been gained about these problems as well.

For an organ of its size and approachability, it is not to be wondered at that a vast amount of work should have been expended in determining the part that it plays in the mammalian economy, but it is surprisingly disappointing that the results obtained in many directions have not been more conclusively indicative of its functions. The frequency of contradictory conclusions—not uncommon in any major topic of medical science—should lead, not to the dismissal of a seemingly unprofitable subject, as several writers have intimated recently, but rather to the careful examination of the available data by those interested in the subject. Such an appraisal will show that many positive facts of value are known about this elusive organ, and should stimulate to further search and to the clearing up of contradictions, which we know must be only apparent. Coincident with our 14 years of study of splenic problems at the University of Pennsylvania, there have appeared in the Biochemische Zeitschrift numerous articles on the subject from Asher's

laboratory in Bern under the general title of Beiträge zur Physiologie der Drüsen. Unfortunately, as I shall have occasion to note in later sections, our results have differed from his in several particulars. In some cases we think we have detected differences in method of attack, which explain the discrepancies; in others they remain unexplained, but in any case tribute must be paid to the tremendous amount of work accomplished at Bern.

1. HISTORICAL AND GENERAL. As it has been known since ancient time that the spleen could be removed without fatal consequences, this negative method of approach has always been a favorite one. The story of splenectomy has been told elsewhere (Krumbhaar), so that a few words will suffice here. It is often repeated that the ancients removed the spleens of runners to make them more speedy ("courir comme un deraté") and the practice is thought to have been due to the erroneous belief that the speedy giraffe had no spleen. Support of this theory has unexpectedly been produced in recent times from a reliable source. Macht and Finesilver found that the muscular integration of splenectomized rats was improved and that over a known length of cotton rope, their "running speed was certainly shortened," but offered no explanation for the improvement found. The possibility must not be overlooked that enlarged pathological spleens undoubtedly were common in ancient times, and the thread of truth in the ancient belief was perhaps due to the fact that runners probably would be speedier if relieved of a big chronic malarial or syphilitic organ. That the spleen was not necessary to life has not always been admitted; Guy de Chauliac, for instance, considered that the spleen's "office is necessary to the whole body as being another liver (as several have said)." Such conjectures would hardly deserve mention, were it not that modern authors still consider it necessary to assert the contrary.

There is little to detain us in the various conjectures about the spleen before the middle of the 19th century. Its swelling in certain acute infections was apparently recognized by the Greeks, but the ubiquity of malaria, even in classical times, here clouds the issue and "lien magnus" was quite impartially ascribed as the cause of bad breath, spongy gums, indolent ulcers, and so forth. A voracious appetite after removal of splenic function, an idea which still crops out occasionally in the literature, seems to have been first observed, together with polyuria, by Malpighi and this latter also by Brunner. These are Malpighi's words: "In a dog of yet tender years a wound was made in the left hypochondrium, and the blood vessels of the protruding spleen and

attached omentum were ligatured with a thread close to the hilus of the spleen; everything was presently replaced in its former position, the peritoneum and the muscles were sutured and the skin loosely united. After a lapse of a few days the wound had healed. After some weeks the animal was strong enough to perform with enjoyment all its proper functions; so long as it lived no trace of any interference with health could be observed. Having become more hungry than before, it took its meals eagerly, devouring bones and food of all kinds. One thing only I observed, namely, that it made water abundantly and most frequently, in fact continually. Though all dogs are continually doing this, it seemed in this respect to outdo all its fellows. Its habit of body was in every respect sound; indeed it became fat, and in other respects, in quickness and alacrity it equalled its fellows" (Foster).

Post-mortem examination showed an atrophy of the spleen, but hardly any other abnormality.

Although supported by the work of Dupuytren, Schultze and others, unfortunately more recent studies have failed to confirm these views (Mosler). A splendid history of the spleen, with the early ideas as to its development, human and comparative anatomy and physiology can be found in Henry Gray's book.

Toward the latter half of the 19th century with the improvements in histological technique, it had become established: 1, That the spleen was not necessary for life and that after removal its functions were apparently taken over by other organs, such as the bone marrow; 2, that from the white pulp surrounding the arterioles (Malpighian follicles) it was concerned with formation of some of the colorless corpuscles of the blood (Virchow); and (3), that it could select various particles from the blood stream, including erythrocytes (Ponfick) and was therefore concerned with blood destruction (Kölliker).

It was also known that the spleen exhibited certain peculiar rhythmical contractions, but this like other numerous observations can better be taken up from this point on seriatim. While it might be possible to consider practically all splenic phenomena from the three points of view, its peculiar anatomical structure, its high content of reticuloendothelial cells and its hemopoietic functions (both direct and in relation to related organs), it is felt that the eight subdivisions used in this article will present a rather complicated subject in a manner easier of comprehension to the average reader.

Variations in relative size of spleen in explaining contradictory experimental findings: An important item for consideration in the comparison

of the results of investigations on splenic function is the size or weight of the spleen relative to the body weight of individuals of various species, even when attention is confined to the class of mammals. Splenectomy, which has played such an important part in investigating splenic function, cannot be expected to have an equal or even comparable effect in two species where the ratio of spleen and body weight may vary from 400 to 500 per cent. Thus it has been found (Krumbhaar and Musser) that whereas in man and the dog the spleen averaged 0.25 per cent of the body weight, in monkeys and guinea pigs, it averaged 0.13 per cent, and in rabbits (and cats) only 0.05 per cent. Gray found that the spleen was relatively biggest in mammalia and especially the bat, which from this point of view should make most desirable material for study. Considerable variation may occur in individuals of the same species, as is shown by Wolferth's obsrvations on rats, where of eight with abnormally large spleens splenectomy proved fatal to seven. In Edentates Retterer found that the relative size of the spleen varied considerably in various types, as it may even in individuals of the same species and was probably in inverse ratio with the other hemopoietic organs. As the spleen in many of its functions forms perhaps the most important part of the reticulo-endothelial system, such variations may be balanced by equivalent changes in other parts of the system, and as I have shown elsewhere, the lost function can be easily compensated for by other parts of the system. It should hardly be necessary to point out that many of the apparent contradictory results found after splenectomy by investigators using different species of experimental animals are probably to be explained partly on these grounds. The frequent failure of the splenectomized rabbit to develop anemia is a case in point and emphasizes again the growing opinion so aptly expressed by a British physiologist that "the hutch rabbit is a rotten little beast anyway." In Weiskotten's diphasic leukopenia in rabbits after benzol injections, the practical absence of any influence of splenectomy likewise argues that the function of the relatively small spleen is unimportant or quickly taken over by other organs. Orr's, and very recently Mole's studies, however, show that even in rabbits splenectomy at times is followed by anemia.[1]

The difficulty of correlating results in different species is greatly increased if classes below the mammals are studied. Thus the erroneous deductions from Minkowski's extirpation of the livers of geese were

[1] An important cause of failure to get results in experimental splenectomy is the frequent occurrence—as high as 75 per cent in the rabbit—of accessory spleens, which have not been removed coincidently with the main organ.

apparently due to the fact that birds' livers contain a bigger proportion of the reticulo-endothelial apparatus than do those of other orders. Similarly in Jordan's studies on frogs, it must be remembered that the spleen is the chief hemopoietic organ (the kidney having this function in the larva) and that apparently regeneration of the spleen regularly follows splenectomy.

Another important item in the evaluation of splenic functions is the age of the individual under examination. Careful studies by Gross have shown that the volume of the spleen as a whole and of its various components follows a definite curve in the life of the individual. It is most active in the young and normally becomes smaller and more fibrotic with age, so that in man Gross considers that it is largely passive after 40. Marine's work tends to confirm this view as do Luckhard's observations that splenectomized kittens usually lost weight and died young. Investigators of the relation of the spleen to various functions in animals should therefore take into account the age as well as the species of their experimental animals, and it is very probable that failure or inability to do this has constituted an important factor in the contradictory results which we hear so frequently emphasized.

One influence of environment on the relative weight of the spleen is shown by Gueylard's work on fresh and salt water fishes. Not only does the spleen of the salt water fish weigh noticeably less in proportion to the body weight, but if the eel, for instance, is moved from fresh to salt water, the weight of the spleen is diminished.

2. Embryology and anatomy. *Embryology.* Although there is still a difference of opinion as to whether the spleen is derived wholly from the dorsal mesogastric mesenchyme or from the mesothelium as well, its anlage can be recognized in the human embryo of the 5th week as an elevation on the dorsal side of the mesogastrium. This is composed of a thickened mesenchyme, vascularized from the mesenteric artery, covered by a thickened mesothelium, from which cells migrate into the subjacent tissue. The mesenchymal tissue is later broken up by blood vessels and ingrowth of trabeculae from the capsule to form the reticular tissue and cells of the pulp cords. The Malpighian follicles are formed by the infiltration of lymphocytes into the adventitia of the arteries and the sinuses from dilated veins.

The formation during fetal life of erythroblasts and leucocytes from reticular and endothelial elements of the spleen is taken over by the bone marrow at about the time of birth. The close interrelationship of the several units of the hemolyto-poietic system, however, is evidenced

by the ease with which the spleen, like the liver, can take over either or both of these functions in adult life if the necessity arises.

Anatomy. The peculiar anatomy of the spleen bears such an integral relationship to some of its functions that it must be considered in some detail in order to comprehend them properly. In the first place, one is struck with the relatively large size of its blood vessels, foreshadowing some of the reservoir functions of the organ, to be discussed later. Man's spleen, however, possesses but relatively few muscle fibers in the capsule. Lymphatic vessels occur immediately beneath the capsule only, in spite of the large amount of lymphoid tissue in the Malpighian follicles. A plexus of non-medullated nerve fibers in man accompanies the splenic artery and its branches and is known to conduct regulatory impulses to the contractile smooth muscle fibers.

It is in the unique course of the blood through the spleen that two of its important functions are indicated (as a "bank" and a "graveyard"). The branches of the splenic artery, travelling deep in the trabeculae, send off subdivisions through the pulp which soon, as the central arteries of the Malpighian follicles (usually eccentric), traverse this characteristic cylinder of lymphoid tissue. Capillaries are given off which end in the follicle or may connect directly with the venous sinus. Again free in the splenic pulp, as end arteries, the arterioles soon break up into the Penicillia of Ruysch. The branches of this, after passing through the peculiar stopcock stage of the Hulsenarterie (artery with husk), (unique to the spleen), either end as a fenestrated end chamber (through which blood may pass to the pulp), or connect directly with a venous sinus. The sinus, which has a peculiar structure roughly comparable to barrel hoops enclosing fenestrated staves, also may receive blood components either through its fenestrations or by tube-like branches draining directly from the pulp; thus including the whole pulp in a cavernous like mechanism. From the sinuses the blood streams are collected into the veins, at the edges of the trabeculae and thus out to the main splenic veins. Thus this scheme contemplates at least three ways in which arterial blood passes to the veins, instead of by the usual capillary connections. While it may not be accepted by all investigators in all details, it is obvious that these alternate routes and the controllable shunts through the pulp, offer splendid opportunities for storage and cellular metabolic mechanisms. In fact clinical evidence indicates that at times the backwaters thus afforded may become a positive disadvantage. The improvement that often follows removal of the chronically enlarged spleen of syphilis or malaria, after medicinal measures

have failed, seems to be due to the fact that the backwater has become a cesspool of offending micro-organisms unreachable in potent amounts by the drugs in the circulation.

A few words about the cytology of the splenic pulp. In the Malpighian follicles, the grouping of large pale cells (lymphoblasts) in the centre

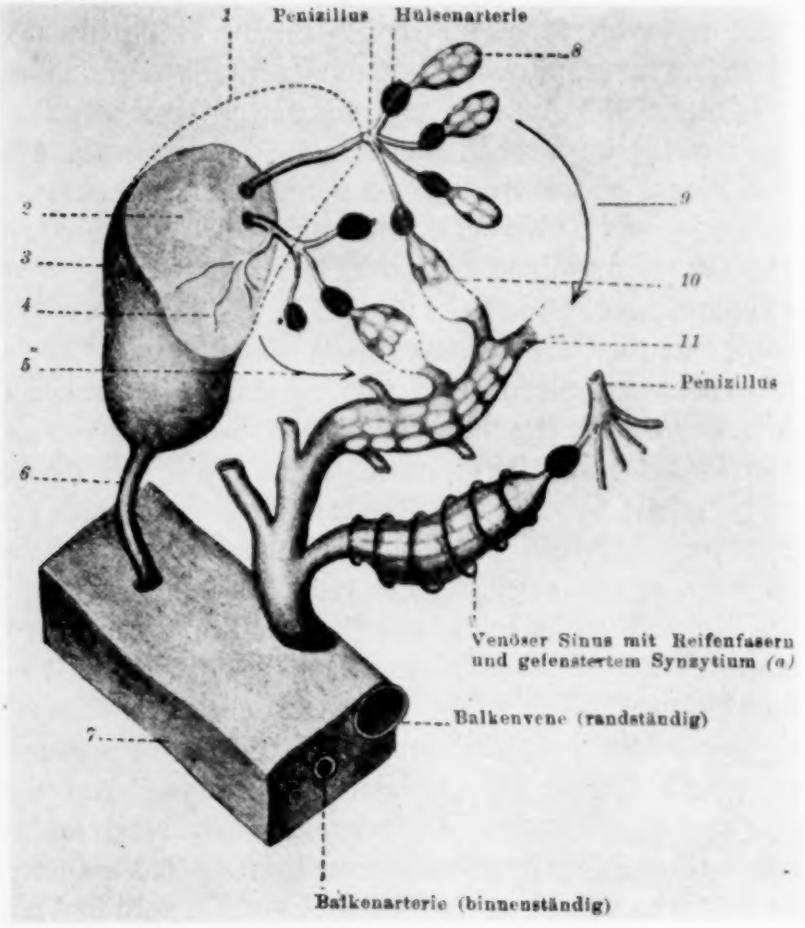

Fig. 1. The vascular system in the spleen. H. Braus' Anatomie der Menschen. *1*, contour of Malpighian follicle completed; *2*, "germ centre;" *3*, Malpighian follicle; *4*, capillary ending in the follicle; *5*, a shunt from artery to sinus; *6*, artery passing through splenic pulp; *7*, a trabeculum; *8*, oval shaped "blind" arterial chamber; *9*, course of blood from fenestrated end chamber to open end of sinus; *10*, another possible course by open passage through pulp; *11*, free communication of sinus with pulp.

with not infrequent mitoses, has long caused these areas to be called "germinal centres," with the corollary idea that the ripening lymphocytes are pushed to the periphery of the follicle till they migrate into the strands of the red pulp (Cp. Bunting's red and white islands in the

bone marrow). To others, however (Latta, Maximow, Mottram) they are rather endothelial cells, capable of phagocyting lymphocytes or bacteria in certain acute infections. The correct answer possibly is that the centres contain both types of cells in varying proportions under different conditions and are concerned therefore in both these functions.

In the pulp are many types of cells: beside the supporting reticulum and the sinus endothelium, now so prominent as important elements of Aschoff's reticulo-endothelial system, are the closely related pulp cells or splenocytes, also of tissue cell origin, and the various blood cells that have left the vascular channels and are wandering, perhaps aimlessly, through the pathless forest. Thus one customarily finds erythrocytes, polymorphonuclears, small lymphocytes, platelets and intracellular and free (extruded?) pigment. The significance of the presence of these cells will be discussed later. As the reticulo-endothelial cells play such an important part in several phases of the spleen's activity, a few extra words about them may be pardoned. Known variously as clasmatocytes (Ranvier), histiocytes (Aschoff), endothelial leucocytes (Mallory), in the liver as Kupffer cells, and as the monocytes of the blood stream, they are found widely scattered through the body, but especially in the pulp and lining the sinuses of spleen and lymph nodes. Endowed with marked phagocytic ability (the scavengers of the body) they are intimately concerned with the disposal of effete erythrocytes, bacteria, foreign pigment and similar deleterious matter. Recently Cunningham, Sabin and Doan have differentiated two types in the rabbit's spleen, one from the monocytes of the blood stream (mesenchymal in origin) and the other, larger and more active in phagocytosis, from the sinus endothelium.

3. Contractility and reservoir function. It has been known for many years that at least two types of rhythmical changes in splenic volume occur, in addition to the Traube-Hering waves—1, a slow expansion and contraction, supposed to be related to digestive functions, and 2, a more rapid systole and diastole with intervals of about a minute, which Roy found in dogs and cats and considered a "specific circulation, ... carried on chiefly if not exclusively by a rhythmic contraction of the muscles" of capsule and trabeculae. Though modifiable by various nervous stimuli, Roy considered this rhythm more or less independent of the arterial pressure, whereas Schäfer and Moore, who confirmed the existence of this rhythm, found that the spleen volume "is extremely responsive to all fluctuations in the general blood pressure." In the light of the recent studies of Barcroft and his colleague at

Cambridge and the University College, London, these earlier investigations acquire renewed interest and importance.

As the Cambridge studies, which are still in progress, constitute one of the most important recent contributions to spleen physiology, they will be considered in more detail than is elsewhere possible. It seems that a probable "bank" or reservoir function of the spleen of significant magnitude was first suggested to Barcroft by the need for explaining the relatively rapid increase in blood volume and hemoglobin estimations, found both clinically and experimentally, on the subject being submitted to higher temperatures. (This function, by the way, was clearly indicated by Henry Gray in his book of 1854.) The possibility of such a function would readily suggest itself to anyone familiar both with the peculiar structure of the spleen, with the experimental proof of its contractility and the rapidity with which it may enlarge in various clinical conditions.

It was also found by Barcroft and his associates that in rats breathing 0.06 to 0.1 per cent carbon monoxide, the poison accumulated in appreciable amounts in the peripheral blood, but only much later in the spleen. The same held true with rabbits and if they were returned to fresh air, the carbon monoxide disappeared from the peripheral blood more quickly than from the spleen. It was therefore concluded that when the animals were quiet the rhythmic contractions were not sufficient to maintain gaseous equilibrium under these conditions (Cp. seclusion of spinal fluid from certain factors influencing the general circulation), the carbon monoxide possibly being kept from the spleen by carbon monoxide stimulation of the central nervous system. Under conditions of strenuous exercise, however, these differences disappeared and the spleen quickly attained the same percentage of carbon monoxide as the peripheral blood.

This blood reservoir function of the spleen is obviously of value to the individual in a variety of normal and pathological conditions. Visual proof of its activity during exercise and in various forms of experimental injury has been afforded by an ingenious method of affixing small metal rods to various crucial points of the spleen surface and estimating the changes in volume of the organ by the position of the rods as indicated by x-ray photographs made before and after the experiment. With a surprisingly small margin of error, subjective bias being effectively excluded, it was found that not only is the living spleen from two to four times as heavy as the dead spleen, but that very considerable shrinkage of the spleen occurred in various conditions when tested both

in rats, rabbits and guinea pigs and cats. In cats, which have very "muscular" spleens, it was estimated that one-sixth of the blood volume or one-third of the number of red corpuscles could be expelled.

This being true, it was inferred that "were the organism presented with an issue which made its life depend on the amount of hemoglobin it could produce, a splenectomized animal should die, when an animal possessed of a spleen should survive." Splenectomized guinea pigs in an atmosphere of coal gas proved the correctness of this inference, while it is interesting to note that splenectomized guinea pigs, exposed to increasing concentrations of hydrocyanic acid—whose fatal effect is not connected with hemoglobin—survived as well as their fellows (see section 6). Thus although, as is so often repeated, the spleen is not necessary to life, here is one instance where it is of distinct use to its possessor, in a function which could with difficulty be compensated for by other organs.

4. Relation of the spleen to blood formation. Investigation of the part that the spleen plays in the formation of blood cells of various kinds has been chiefly directed toward: *a*, the microscopic evidence of the presence in the spleen of immature blood cell forms under normal and pathological conditions; *b*, the relative counts in the blood going to and from the spleen; *c*, the blood picture following splenectomy and the changes in the other organs of the hemolytopoietic system following interference with splenic function.

A. Microscopic evidence. Under this heading, reference has already been made to the generally accepted belief that hemopoiesis regularly occurs in the embryonic mammalian spleen (Thiel and Downey, Takagi) just as it does in the liver. In fact, v. Melczer believes that it furnishes leucocytes of various kinds throughout life to the circulating blood, chiefly lymphocytes and large mononuclears up to the third year, after which the neutrophiles assume the leading position. It would be desirable, however, to have more precise information as the relative importance of the spleen in hemopoiesis, and the time and manner and degree of its relinquishment to the bone marrow in the various species, as Takagi has done for the dog.

Reversion to or persistence of this myeloid function during adult life has been more extensively, though still inadequately studied. A considerable number of clinical post-mortem observations, usually where there has been a severe chronic anemia, or extensive injury to the bone marrow (Meyer and Heineke, Donhauser and Wade) have shown in one type numerous erythroblasts, myelocytes, giant cells, etc., in splenic

sinuses and pulp; and in another, large areas of tissue indistinguishable in every detail from active bone marrow (myeloid metaplasia). In the former group it is improbable, though impossible to deny absolutely, that these cells have been swept in from other sites; in the latter, without stopping to discuss the way in which the tissue is formed, it is obvious that active blood cell formation is in progress. As Bunting, Morris and Itami all showed a number of years ago, this result can also be produced experimentally with agents destructive to blood cells, such as pyrodin and phenylhydrazine. In rabbits, Itami regularly secured extra-medullary blood cell formation in the spleen in eight to ten days by this means, while the same phenomenon appeared in the liver more slowly and less constantly. It is important to note (though for reasons still unknown), that an equally severe anemia caused by withdrawing blood failed to produce this result, unless defibrinated blood were simultaneously injected. Dominici, to be sure, had previously got a myeloid change in the spleen of rabbits subjected to repeated bleedings. The failure of Weiskotten and his associates to obtain "myeloid metaplasia" in rabbits whose leukopoietic function has been depressed by benzol, is thus explained by Itami's results.

The considerable formation of lymphocytes by the Malpighian follicles of the spleen might almost be assumed to take place normally, as they constitute with the lymphoid tissue of the intestines two of the chief sites of lymphoid tissue in the body. Fortunately, however, there exists evidence in support of this assumption. Downey and Weidenreich showed that lymphocytes are supplied to the circulation, not only from the Malpighian follicles but also from the pulp tissue, the only difference between the spleen and lymph nodes in this respect being that in the absence of lymph channels in the spleen, the lymphocytes are furnished directly to the splenic vein, and V. Melczer's work has already been referred to. Furthermore, R. L. Dixon showed that following splenectomy, the lymphocyte count in the thoracic duct was lowered even when the thoracic duct cell count had been raised by pilocarpine injection. Frey's functional test of the spleen (increased lymphocytosis following injection of adrenalin) is based on this lymphocytogenic rôle. Finding that lymphocytosis failed to appear when adrenalin was injected during the first five months after splenectomy, he concluded that such failure would practically indicate a loss of splenic function, but other authors were quick to point out the theoretical and practical weaknesses of such a test (Oehme). Although all the other formed elements of the blood have been found being formed in the adult spleen

under abnormal conditions, it is not thought by most observers that they are formed there under normal conditions.

b. Relative counts in the blood going to and from the spleen. This has been a favorite method of approach for some time. In our hands, as stated in our book on the *Spleen and Anemia* (p. 198), however, "detailed comparisons of the arterial and venous blood of the spleen offer no evidence to indicate, by the methods used, that the spleen has an important rôle in blood formation, or, on the other hand, that it is appreciably active in blood destruction." This latter point will be treated more in detail in the next section, on account of new data now available. In regard to blood formation, suffice it to say that no adequate evidence has been produced by ordinary methods of blood counting since then to contradict this statement and probably never will be, as the limits of error of these methods are greater than the differences one could hope to find under normal conditions. This applies to the higher leucocytic and red cell counts found by Morris and some of the earlier investigators in the splenic vein, as well as to our own observation of its greater reticulocyte content in five out of seven dogs.

c. The blood picture following splenectomy. The anemia, therefore, that follows removal of the normal spleen in most species of mammals must, I feel, be explained on some other basis. The severity, duration and variation of this anemia have been sufficiently discussed in the *Spleen and Anemia*, as but little further attention has been paid to its study in the past eight years. In Takagi's extensive studies on young puppies the anemia lasted longer than in adults and the increased resistance of the erythrocytes was more marked.

Although a dogmatic insistence on the point would be premature, various experiments tend to show that this transient anemia is caused by a loss with the spleen of some substance which exerts a stimulating influence on the bone marrow; for these reasons (*Spleen and Anemia*, p. 111): 1. The anemia is due to lack of blood formation rather than to increased blood destruction (usual absence of blasts, decrease in reticulocytes, low fecal urobilin). 2. It can be relieved by the administration of splenic extract (which we and others have shown was in a class by itself in raising both the white and red cell counts of normal dogs (*Spleen and Anemia*, p. 93), and which Leake and others have successfully used very recently in combination with bone marrow extract as a stimulant to hemopoiesis). 3. The anemia disappears more or less coincidentally with a bone marrow hyperplasia, which can be considered,

in part at least, to be compensatory in nature (Krumbhaar and Musser). This compensation may at times be excessive (the common biological phenomenon of overswing of the pendulum) as was shown by the occurrence of spontaneous jaundice in some of our dogs, and the occasional clinical report of polycythemia following splenectomy. (It must be recalled that in view of the bone marrow's connection with blood cell destruction, a mere increase in the cell content of the shaft marrow—the usual criterion of bone marrow hyperplasia—does not necessarily mean increased hemopoiesis.) Naswitis found that injections of laked blood increased the blood count of normal dogs, but not of splenectomized dogs. He therefore concluded that the spleen must be stimulated by the injected blood to form substances, which in turn stimulate the bone marrow to increased activity. This still rather intangible bone marrow stimulant was not able in our hands, like thyroid extract in its sphere, to prevent the post-splenectomy anemia of dogs when the raw spleen was fed in adequate amounts. Furthermore in our control experiments on dogs, where ligation of the splenic vein, Eck fistula or transplantation of the splenic vein into the vena cava were substituted for splenectomy, the same sort of anemia was obtained, so that if our splenic stimulant to the bone marrow exists at all, it would seem necessary for it to be activated by passage through the portal circulation to the liver (*Spleen and Anemia*, p. 130). On the basis of such evidence Eddy believes that the spleen produces an internal secretion from material elaborated from the disintegrated erythrocytes which it has removed from circulation, that this internal secretion reduces the resistance of all red corpuscles, thus actually destroying the weaker ones, and that as a hormone, possibly after modification by the liver, it stimulates the erythrogenic function of the bone marrow.

Arguing from an opposing point of view, namely, the rise in the blood picture (in disease or only for a few hours in normal animals) and the appearance of Howell-Jolly bodies of the so-called "blood crises" that sometimes occur after splenectomy, Stradomsky and others have hypothesized an inhibitory action of the spleen on hemopoiesis. We feel, however, that other and perhaps better explanations exist for these phenomena. The early, inconstant and quite trivial rise in the hemoglobin and red cell counts after experimental splenectomies are best explained as a relative anhydremia, and the leucocyte count (always an unreliable element) as a non-specific post-operative effect. The longer duration of the leucocytosis, however, which in dogs we thought characteristic of splenectomy, is harder to explain. In monkeys we found

it much less prominent than in dogs. When persistent it is perhaps due to the merging of the post-operative effect into the compensatory effects in other parts of the hemopoietic system that are discussed elsewhere. We have felt that the late relative lymphocytosis found by us and others (*Spleen and Anemia*, p. 18) was of this nature. Beresow, however, finding that this lymphocytosis is more susceptible to atropin effects, believes it caused by an increased tone of the autonomic nervous system, due to loss with the spleen of a hormone paralyzer. Clinical reports of a rise in the blood picture after splenectomy are easily explained on other grounds. They are found either following rupture of the spleen, with its resultant hemorrhagic anemia to be replaced, or in some form of non-hemorrhagic anemia such as hemolytic jaundice, Banti's disease or pernicious anemia, where the benefits accruing from removal with the diseased organ of an important factor in blood destruction might fairly be considered far to outweigh the loss of the normal stimulant to the bone marrow. In the clinical reports of splenectomy for cysts and conditions other than the above, anemic blood pictures quite similar to those found by us in dogs were obtained.

The appearance of Howell-Jolly bodies (immature erythrocytes) has likewise been usually noted after splenectomy for some of the primary anemias and thus would be explained in the same way. We constantly failed to find them after removal of the normal spleens of monkeys and dogs, though Takagi found them constantly in puppies splenectomized shortly after birth. Recent studies on rats by Waltz, furthermore, tend to show that the appearance of immature red forms after splenectomy is not due to increased bone marrow activity, but rather to delayed cell maturation, thus bringing the phenomenon in agreement with a splenic bone marrow stimulant theory.

Not only does the spleen affect the number of blood cells in the peripheral circulation, but also their quality. Practically all observers are unanimous in agreeing that after splenectomy the red blood cell becomes less fragile (i.e., more resistant to hemolysis by hypotonic salt solution and other destructive agents), (Krumbhaar and Musser; *Spleen and Anemia*, p. 42). This occurs in numerous species studied under normal and pathological conditions (both experimental and clinical) and has been known to persist for many years, especially in the primary anemias. It is undoubtedly a factor in the beneficial results of splenectomy in anemias and in the lessened amount of jaundice that is known to follow the administration of hemolytic agents to splenectomized animals (Joannovics, *Spleen and Anemia*, p. 54). The part that this phe-

nomenon plays in the spleen's relation to blood destruction will be discussed in the next section. In our dog work, we preferred to consider that it was associated with post-splenectomy or other forms of anemia or with the initial regenerative processes in the blood thereafter, but it must be frankly admitted that what scanty evidence there is on this point is divided and definite conclusions impossible. Donati's observations that for at least a month after splenectomy dog's erythrocytes are more permeable to glucose than normal would tend to show that the increased resistance is at least not due to a larger proportion of young cells.

5. Relation of the spleen to blood destruction. This phase of splenic function may like the previous section best be considered under such subdivisions as, *a*, microscopic evidence; *b*, relative appearance of the blood in the splenic artery and vein; *c*, functional changes produced by splenectomy; *d*, disposal of products of red blood cell destruction.

a. Microscopic evidence. The spleen's relationship to blood destruction has received considerable attention for many years (*Spleen and Anemia*, p. 169) on account of the obvious richness of the spleen in blood, but especially since Kölliker and Ponfick called attention to the microscopic evidence that the spleen actively participated in the elimination of erythrocytes and the products of their disintegration together with, as was later found, bacteria and other foreign matter. Not only were occasional fortunate observations made of this occurrence during normal splenic activity, but under many conditions of increased blood destruction (both experimental and clinical), phagocytosis both of blood pigment, fragments of erythrocytes and even whole erythrocytes was found to be constant and extensive (the spodogenous spleen of Ponfick). Details of the cycle of changes produced in the rabbit's spleen during phagocytosis have been described by Addison. Such phagocytosis, to be sure, may also be active in the bone marrow and other organs of the hemolyto-poietic system, especially after splenectomy (*Spleen and and Anemia*, p. 164), and it is generally accepted that the chief rôle therein is played by the large mononuclear cells of the so-called reticulo-endothelial system. The relatively facile compensation for the loss of the spleen in this particular is thus readily explained by the widespread distribution of this important group of cells. Details of current views on the mechanism of red blood cell destruction have been so lucidly put forth by Rous in this journal (1923) that it is unnecessary to pursue them further at this time. Rous' own contribution that fragmentation

of erythrocytes within the circulation is an important early step has received more or less general acceptance and to the degree of bipartition has been found also by Spadolini in amphibia. Jones and Jones, in fact, believe that a large part of the transformation of hemoglobin into bilirubin can normally occur within the blood vessels.

b. The blood picture in splenic artery and vein. The application of these studies to blood cell formation was discussed in section 4. They have played a more prominent rôle in attempting to elucidate the spleen's relationship to blood cell destruction. Thus various authors found fewer or less resistant cells in the splenic vein (*Spleen and Anemia*, p. 87), (though others found the opposite) or even free hemoglobin in quantities measurable by a Sahli hemoglobinometer. Our own experience convinced us, however, that the slight and inconstant differences observed were well within the limits of error inherent to the relatively crude methods of blood examination (or in some cases to technical errors) and were not to be explained by any specific action of the spleen. Subsequent investigations based on blood counting methods fail to suggest a modification of this opinion. Very recently, however, more refined technical methods (spectrophotometric) have permitted Mann and his colleagues to assert that blood coming from the spleen and bone marrow of normal animals contains appreciably more bile pigment (presumably a product of erythrocyte destruction) than does arterial blood or blood issuing from other organs. They are careful to point out, however, that the amounts involved would be quite beyond detection by usual methods of examination. Qualitatively, then, this method of comparing artery and vein, like the microscopic evidence, supports the view that the spleen is one of the organs chiefly concerned with blood cell destruction. Last June also Rich and Rienhoff reported that in four out of ten clinical cases there was more bilirubin—a substance not capable of quantitative estimation in the earlier studies—in the splenic vein than in the splenic artery or the peripheral veins.*

c. Functional changes produced by splenectomy. Such studies, of course in a negative and often indirect manner, also support the view that the spleen is concerned in blood cell destruction. We have seen that splenectomy is constantly followed by an increased resistance of circulating erythrocytes to various destructive agents. The spleen then in some way still unknown (see, however, the later section on fat metabolism) may be presumed to have some action in making them more fragile. This forms the basis of Bottazzi's hemocatatonistic theory; that the

*In a recently splenectomized case of hemolytic jaundice we were unable to detect this change spectrophotometrically.

spleen prepares red blood cells for destruction. Here too belong the observations that following splenectomy the elimination of bile pigment is diminished (Pugliese, Goto), though this has been denied by others, and that following splenectomy there is a lessened tendency to jaundice following the administration of hemolytic agents, an observation to which, as far as I know, no exception has been taken. Pugliese and Luzzatti made similar observations with pyrodin; and while Bodansky, Hartman and Gleckler believe that in their hands splenectomy did not appreciably modify the rapidity and severity of red cell destruction with this drug. Comparison of the blood pictures at the time the drug was given, as far as their published results permit a comparison, shows a definitely greater drop in the controls, if the pre-injection level of the splenectomized animals be taken. The simplest explanation of the lessened tendency to jaundice, that the removal of an organ active in blood destruction causes less blood to be destroyed and therefore less bile pigment, apparently will not entirely suffice, as we found that after the hemolytic poisons the blood counts of the splenectomized animals both reached a lower level than the controls, though the drop was not actually as great, but also they took longer to return to their post-operative levels. Why then do they show a lessened tendency to jaundice? Two important factors appear to be: the influence of the post-splenectomy anemia and the increased resistance of the red cells, each factor reducing the amount of hemlgoobin set free. The most important factor, however, seems to be the purely mechanical question of how the hemoglobin liberated by the hemolytic agent (or bilirubin) reaches the liver. The pigment normally coming at least in relatively large proportions from the spleen directly to the liver by the portal circulation in concentrated form, jaundice is more apt to result. In the absence of the spleen, its function being taken up by other members of the hemolyto-poietic system, the products of blood cell destruction reach the liver more slowly and diluted, so that jaundice is less apt to occur. This theory is strengthened by our observations, previously referred to, on splenic vein ligation, Eck fistula and the modified Eck fistula, where the same lessened tendency to jaundice was obtained (*Spleen and Anemia*, p. 121).

The recent clinical observations that splenectomy has a strong beneficial effect, perhaps actually curative, in certain types of chronic purpura directs attention toward the spleen's relation to blood platelets and blood coagulation. The marked rise in the platelet count in these cases would suggest that the spleen normally destroyed platelets; but comparative counts in the splenic artery and vein and peripheral veins (Holloway and Blackford) fail to bear this out. As in the case of the

erythrocytes, it would seem preferable to search for some regulatory influence of the spleen on the megakaryocytes of the bone marrow (the site of platelet formation). In the case of the purpura effect, the complication added by the fact that normal organs are no longer being dealt with, must also be taken into consideration. As regards coagulation time, Henn (ref. section 7) found it greatly shortened in a splenectomized dog (no platelet counts given), but no material difference in rats and rabbits. In our work (Krumbhaar and Musser) platelet counts proved quite inconstant after splenectomy, though unpublished experiments indicate that both coagulation time is shortened and platelets increased after splenectomy of dogs.

d. Disposal of products of red blood cell destruction. Studies of the rôle played by the spleen in the processes of blood cell destruction have always left a painful gap in the argument, that remind one of the state of the theory of the circulation of the blood before Malpighi's discovery of the capillaries. It was evident that the spleen could and did engulf erythrocytes, that the process of blood cell destruction was disturbed when the spleen was removed and at the other end that bilirubin was not only chemically related to hemoglobin and hematoidin, but fluctuated roughly parallel with fluctuations in red blood cell destruction. The intermediary stages, however, have only been illuminated (and still far from completely explained) in the past few years and even months, and like Malpighi's discovery were largely due to improved methods in technique .

That bile pigment could be formed outside the liver was first demonstrated experimentally by Whipple and his colleagues in 1913 on animals in which the liver had apparently been cut out of the circulation. Though it seemed at one time as if these experiments might be invalidated by the escape of some of the smaller hepatic vessels, they have since been amply confirmed by Mann's ingenious method, which permits a sufficiently long survival of the hepatectomized mammal, to demonstrate bilirubin formation in the absence of the liver, and also by Rich's demonstration that bilirubin can be formed from hemoglobin by endothelial cells growing in vitro. In fact, as Rich points out, in his excellent review of the "Formation of Bile Pigment" in last April's number of this journal, while proof exists that bilirubin is formed outside of the liver (probably by the reticulo-endothelial cells), there is no proof that the liver cells themselves ever form bilirubin.

We may now conclude, therefore, with a fair chance of being right, that no matter what determines which erythrocytes are to be destroyed

or where the process begins (Rous' fragmentation theory), they or their component parts are taken up by phagocytes, where bilirubin is formed, to be delivered by the blood plasma to the liver (in almost indetectable amounts, to be sure, under normal conditions). In this process, the spleen, both on account of its size, its anatomical structure and its upstream relation to the liver, plays an important part.

It is difficult to leave the question of bile pigment formation without a few words about McNee's hypothesis that the columns of liver cells in transferring bile pigment from the vascular to the bile capillaries, may be looked upon as semipermeable inverted test tubes, receiving from the outside the pigment elaborated in the reticulo-endothelial system and perhaps modifying it in passage through the cell to the bile capillary (the lumen of the test tube, as it were). This action of the liver cell might explain the two types of bilirubin found in different clinical conditions as indicated by the direct and indirect reactions of the Van den Bergh test.

Clinical and pathological evidence strongly supports the belief that the spleen plays an important part in the bile pigment preparation and that this function may become perniciously active in certain diseases (Eppinger's hypersplenism). In hemolytic jaundice, *par excellence*, where blood is being destroyed in very large amounts, removal of the spleen is promptly followed by an astonishing reduction in the amount of blood destruction with the practical cure of the patient. In Banti's disease, also, permanent improvement follows removal of the spleen. Even in pernicious anemia splenectomy usually brings prompt improvement, especially in the matter of relieving the excessive blood destruction; but here unfortunately the gain is only temporary, as the other members of the hemolyto-poietic system soon take over the spleen's activity in destroying an excessive amount of blood. The answer as to why this should occur in the case of pernicious anemia and not of the other diseases would probably go far toward explaining the nature of this baffling disease.

6. Relation of the spleen to immunity. *a. Antigenic.* This is undoubtedly one of the fields in which any considerable participation of the spleen has been most called into question and superficial investigation permits an array of a fairly well balanced list of authorities on either side of the question. Closer examination of the problem, however, seems greatly to lessen the apparent contradictions and without allowing anything like a final word on the subject, to permit a fairly consistent and valid statement of this phase of the spleen's activity.

For many years the spleen has been associated with resistance to bacterial infections on both clinical ("acute splenic tumor") and experimental grounds. Wyssokowitsch in 1886 showed that bacteria injected intravenously disappeared from the blood in a few hours, to be found in great numbers in the spleen. Bull has determined this quantitatively and finds that after injection of 200 million bacilli, 2 million colonies will be prepared by the spleen, as compared with 1,600,000 by the liver, 100,000 by the lung and 1,000 by a mesenteric lymph node. The number of colonies in other organs was quite insignificant.

For more than a quarter of a century, too, experiments have been produced to show that the spleen was an important (never the sole) site of antibody formation and therefore intimately concerned with resistance to infection. Bardach's[2] success in killing splenectomized dogs with anthrax bacilli in doses insufficient to kill normal rabbits, and Tizzoni and Cattani's short communication on the failure of splenectomized rabbits to develop immunity to tetanus were among the first, if not the first, in this field; while Pfeiffer and Marx's more detailed investigations on the site of origin of immune bodies in rabbits were also important in calling attention to the activity of the spleen, but also the bone marrow and to a lesser extent the lymph nodes and lungs, in protecting against injections of cholera bacilli. These authors observed that this protection largely disappeared if attempts to stimulate antibody formation were made immediately after splenectomy; but in rabbits splenectomized months before, antibody formation was unimpaired, i.e., the spleen's rôle had been taken over by related organs—a familiar statement in this article. Wassermann also found that in his experiments the spleen played a rôle in pneumococcic immunity.

The argument was hotly pursued and for a decade the "Noes" seemed to have the advantage. Deutsch (1898), Rath (1899) and others were unable to find as many antibodies in the spleen as in the circulating blood and Kurlow (1889), Benario (1894) I. Levin (1902) ("not indispensable"), Takuschewitch (1904), Kraus and Schiffman (1906), Szokalski (1900) and still others failed to find that splenectomy materially influenced antibody formation. These investigators used several different species of animals, attempted immunization soon and late

[2] Bardach's paper is said to be also of interest as an early example of "blockage" experiments, which have again become popular with the so-called reticuloendothelial system. I have not been able to find in it any basis for this assertion, and suspect that confusion may have arisen from the double meaning of the French word "Charbon" (i.e., anthrax and carbon).

after splenectomy, with various organisms and seemed to have presented a strong case against any significant rôle for the spleen in this regard.

Later and usually more careful experiments, however, seem now to have swung the argument definitely back to the side of the protagonists, i.e., under suitable conditions, the spleen can be shown to play a definite and important rôle in antibody formation and resistance to infection. Thus Luckhardt and Becht demonstrated that under their conditions the dog's spleen can partly fix injected antigens and that splenectomized dogs do not produce hemolysins, hemagglutinins or hemopsonins as rapidly nor in as high concentration as the controls. Benjamin and Sluka found that rabbits were able to produce fewer precipitins for beef serum after radiation of the splenic area. In Morris and Bullock's careful and extensive studies of the resistance of several hundred normal and splenectomized rats to rat plague, it was found that absence of the spleen was associated with a much higher (80.5 per cent, 84.7 per cent, 87.5 per cent and 87.5 per cent) and quicker mortality than in the castrated controls (38.9 per cent, 29.2 per cent, 22.7 per cent and 12.5 per cent). Hektoen's studies on rats, continued over a period of 5 years, showed that production of lysins against sheep's blood was influenced by splenectomy, as well as by radiation, but only if done about the same time as the antigen is injected and in suitable doses. When antibody formation was well under way or if too large an amount of antigen was injected, splenectomy had little or no effect. Precipitins also were found by Bieling to be lessened in rabbits, following radiation of the splenic area in suitable doses and Wohl very recently has reported that hemolysins are likewise reduced in rabbits and guinea pigs by large doses of x-ray. Motohashi, too, had found that splenectomy profoundly modified the production of specific hemolysins in rabbits, but that even these marked results could be masked by sufficiently large doses of antigens. Bieling further found that splenectomy during injections of antigens distinctly lowered antibody formation, but especially if combined with attempted "blockage" of the rest of the reticulo-endothelial system. Antibodies, therefore, might be looked upon as "incretes" of this system. In certain infections, however, such as tetanus, splenectomy seemed to be of little importance, an observation supported by the fact that the spleen did not swell after the injection of soluble toxins. Perfusion experiments on excised spleens also show that bacteria are ingested by the splenocytes (and polys as well), (Ozaki), and that sheep's spleens, much like guinea pig's, will bind a certain amount of tetanus toxin and normally contain erythrocyte agglutinins, which the liver does not (Hahn and V. Skramlik).

There is evidence, on the other hand, to be sure, that in tuberculosis removal of the spleen increases resistance to infection. Thus Lewis and Margot found relative but definite increase in resistance to bovine tubercle bacilli by splenectomized mice; Murphy and Ellis similarly and by finding greater susceptibility to infection of animals irradiated with lymphocyte destroying doses, concluded that lymphocytes were the important agents in combating tuberculosis. The increased resistance of the splenectomized animals therefore was associated by these authors with the increased lymphocyte count frequently found after removal of the spleen. Morton, on the other hand, found that radiation of the whole animal reduced guinea pigs' resistance to tuberculosis, which was in turn denied by Kellert, the discrepancy perhaps being explainable by differences in dosage and by differing periods between radiation and inoculation. If this increased resistance to tuberculosis by the splenectomized be true, and in some of these studies there were extenuating circumstances, then a different activity of the spleen (its lymphoid rôle) may be called into question and the results not necessarily contradictory to the work previously quoted, which concerns the reticulo-endothelial elements of the spleen.

With the probable exception, then, of those diseases which are opposed by lymphocytic resistance, the balance of evidence strongly favors the view that the spleen plays an important rôle in the production and storage of immune substances. This importance, however, probably varies for different disease conditions; the rôle in any case can quickly be taken over by other organs in the absence of the spleen; and at any time can be masked if sufficiently large doses of antigen are used. This in turn indicates that, as with one of the chief factors of blood destruction, it is the reticulo-endothelial system that is here concerned and that the spleen's importance is due to the fact that it is the chief habitat of this system.

b. Tumor formation. The relative freedom of the spleen from clinical tumor metastases naturally raises the question as to whether it is not especially concerned with the biological resistance of the organism to tumor formation, a condition accepted by some cancer investigators and denied by others. Murphy's studies of chick embryo tissue would certainly suggest that the spleen (and to a less extent the bone marrow) provides a defensive mechanism against the development of foreign tissues that is not possessed by other organs. If chick embryo was inoculated simultaneously with a fragment of rat sarcoma and adult chicken kidney, for instance, Murphy found that the two grafts grew

and intermingled happily together. If, however, spleen were substituted for kidney, the embryo resisted the growth of the foreign sarcoma, which underwent rapid retrogression.

The factors involved in biological resistance to tumor formation are much less clearly understood, for various reasons, than are the factors of bacteria immunity: tumor colonies are less adaptable to laboratory study than are bacteria, comparison of virulence of various tumors is difficult and even individual tumors vary greatly in virulence in different stages of existence. It is not to be wondered at, then, that different investigators have reported incompatible results from their studies. Thus Brancati found that tumor grafts grew much less luxuriantly in the spleen than in other organs, and that weakly growing tumors did much better in splenectomized than in normal animals (also Oser and Pribram), and that splenectomized animals were more difficult to immunize against subsequent tumor grafts. In the same way Beidre found that splenic extracts were much more efficient than those of other organs in immunizing mice against subsequent inoculations, a result also obtained by Frankl with rat sarcomata and also by Biach and Weltman who believed that the spleen contained specific substances which destroyed tumor cells. Apolant, too, found that active resistance to tumor inoculation can be inhibited or at least diminished by splenectomy. More recently Lazarus-Barlow and Parry found that the spleen offers greater resistance to grafts of Jensen's rat sarcoma than does any other organ, and that after inoculation of sarcoma tissue into the spleen, general resistance to tumor grafts is raised more than by similar treatment of any other organs. On the other hand Pitzman failed to find any immunizing power in splenic autolysates and explains the positive results of others on the basis of infections. Bullock and Rohdenberg working with various tumors in rats and mice failed to find that splenectomy exerted any appreciable influence upon immunity against transplanted tumors. In their hands, removal of the spleens of normal rats, either before or after inoculation with tumor, had no effect on the percentage of takes or of spontaneous absorptions, the degree of resistance to or on the persistence of immunity induced by receding tumor grafts. While it seems preferable, therefore, to leave this question open, it should be noted that some of the conditions imposed by these authors were very rigorous, for instance, in two of the four experiments quoted in their last paper only one graft "took" in the splenectomized mice, but only one in the controls. It is easily conceivable that conditions for takes may have been so unfavorable that the resistance supposedly lost with the spleen may

have existed but not been of sufficient magnitude to be apparent under the conditions imposed. The more recent negative results of Dobrovolskaia are too undecided and fragmentary to warrant further consideration. With the great activity now being displayed in experimental cancer work, and the number of new methods available, it is to be hoped that conclusive evidence will soon be produced concerning the rôle of the spleen toward tumor formation.

7. Relation of the spleen to digestion and metabolism. *Digestion.* The influence of the spleen upon digestion has been so long debated that the accumulated evidence pro and con is too voluminous to be presented in this article. A short discussion of some of the earlier metabolic work is given in *The Spleen and Anemia* (p. 181), and a much fuller historical review in Inlow's series of papers in the American Journal of Medical Sciences. Richet's observations that splenectomized dogs need more nourishment than the controls and that if they fail to get it, they deteriorate and often die, are usually quoted on the positive side; to which may be added his further experiments of three years ago, showing that fasting dogs suffer more ill effects when deprived of their spleens than do normal dogs. Ducuing and Soula also found that young splenectomized dogs and rabbits grew more slowly than their controls.

In the recent literature this can be counterbalanced with Smith and Ascham's studies on splenectomized rats under conditions of diet and selection of animals more rigidly standardizable than is possible in dogs. They found "no indication whatever of an increased appetite as a result of removal of the spleen" and no evidence of a variation from the normal rate of growth, but unfortunately give no tables. (They also failed to find any post-splenectomy anemia.) Lombroso's and more recently Mollow's studies of dogs with gastric and duodenal fistula before and after splenectomy (like Inlow's experiments) failed to show any change in the gastric and pancreatic juices other than some alteration in volume. The majority of the recent work, then, has tended to deny the earlier observations that the spleen was directly concerned with digestion.

The possible deleterious effect on growth of removal of the spleen of the young has received more or less attention in connection with nutrition studies. Aside from a few positive observations (mostly from the French school, but see Luckhard, p. 158), properly controlled experiments have been negative. (Asher and Grossenbacher, Takagi, Henn.) Neither can I find any substantial basis for the opinion held by some surgeons that the life expectancy of splenectomized patients is shortened any more than concomitant disease might indicate.

Turning now to various special fields of metabolic study, we again find that an immense amount of work has been done, especially in recent years from Leon Asher's laboratory, but although advances have undoubtedly been made, they hardly seem as yet commensurate with the energy expended.

Basal metabolism. Verzar failed to observe any changes in basal metabolism after splenectomy in curarised dogs. Danof, however, found in Asher's laboratory that basal metabolism was increased in rats after splenectomy; later opposite results were obtained by Kada, Doubler and Asher (as well as by Marine and Baumann), but the explanation was next thought by the Bern School to have been furnished by still further investigation by Takahashi and Bernet, both of whom found that if the respiratory metabolism were studied under conditions of oxygen lack, or during ingestion of thyroid extract (i.e., conditions making greater demands on the efficiency of the animal), then splenectomy was followed by greater increases in basal metabolism than in normal animals. The very latest report, however, from this laboratory seems to complicate the matter still further—Nakayama's study of two dogs indicating that the increased respiratory exchange brought about by a controlled meat diet is less marked after splenectomy than in the controls, forcing the rather lame deduction that splenectomy affects basal metabolism only as it affects the state of the liver! Granted the difficulties interposed by the complexity of the problems and the technical methods involved, it is unfortunate that it has been so often clouded with immature conclusions from dubious results, based on inadequate material.

Nitrogen metabolism. From the aspect of nitrogen partition and elimination we, like other observers, failed to find any disturbances after the removal of dogs' spleens (*Spleen and Anemia*, p. 194). Mendel and Jackson also failed to find any relation between splenic function and purin metabolism, in spite of earlier claims that the spleen was concerned with the formation of uric acid.

Carbohydrate metabolism. Studies on the relation of the spleen to carbohydrate metabolism, infrequent to be sure, seem to negate any great influence in this direction. Austin and Ringer did not find that splenectomy modified in any way the course of a dog's glycosuria caused by phlorhizin; Verzar found that intravenous injections of dextrose and soluble starch were promptly utilized by splenectomized dogs. More recently, to be sure, Togawa in Asher's laboratory contradicted Verzar's work and found that splenectomized rabbits contained more glycogen

while fasting, but on resumption of feeding did not prepare new glycogen as well as their controls. Splenectomy, however, failed to change the blood sugar content of the blood of Togawa's rabbits. If the spleen, then, does have any effect on carbohydrate metabolism, it is probably through its influence on other organs and its unquestioned demonstration will have to await future investigations.

c. Fat metabolism. The importance of the spleen's relation to fat metabolism was stressed by Eppinger, who found therein an explanation of the beneficial results that follow splenectomy in certain anemias, such as hemolytic jaundice and pernicious anemia. Observing, with King, that there was an increase in the total lipoids and the unsaturated fatty acids in the blood, in conditions both clinical and experimental associated with increased blood destruction, and that these elements were diminished by splenectomy, these authors concluded that there was a parallelism between the degree of hemolysis and the amount of unsaturated fatty acids in the blood and that the amount of cholesterol, an antihemolytic substance, under certain conditions, was also significant. Our own studies on normal dogs failed to show any marked disturbance in fat utilization after splenectomy (*Spleen and Anemia*, p. 190). King's observation that splenectomized dogs held transfused blood longer than their controls seemed also to him a support for this belief.

Unfortunately for King's point of view, Bloor has recently found that simple withdrawal of blood from dogs (i.e., no hemolytic factor) will cause an increase in the percentage and degree of the unsaturated fatty acids in the blood. He suggests as a possible explanation that either the change is due to a loss with the erythrocytes of a factor in fat metabolism, or that in the bone marrow hyperplasia that follows anemia, fat is forced out of the marrow into the circulation. In any case, he emphasizes that the increase in unsaturated fatty acids is the result, not the cause, of the anemia. Even if these deductions prove to be correct, we still have to reckon with the fact that splenectomy has been found to reduce these values, even though splenic activity may not have been responsible for their abnormal increase.

Studying plasma and corpuscular cholesterol in various types of clinical anemias, MacAdam and Shiskin, found that the plasma cholesterol was equally reduced (the corpuscular fraction being much more constant) in pernicious and in secondary anemias, but that it was considerably increased after splenectomy (also Siegmund). They did not, however, believe that this was related to any change in the unsaturated fatty acids or that an abnormally high per cent of cholesterol as ester

was a factor in the production of anemia. Rosenthal and Holzer take still a different stand: they find no characteristic changes in cholesterin, cholesterin ester and iodine count in severe anemias, and while plasma cholesterin seemed to them to be increased after clinical splenectomy, the unsaturated fatty acids showed no appreciable change. Although an increased plasma cholesterol after splenectomy seems to be one of the few undisputed items of these studies, Abelous and Soula believe that the spleen has a cholesterinogenic function. They found that dog's serum collected after a meal rich in fats increased its cholesterol content for several days, while the fatty acids diminished. The serum of splenectomized dogs, however, behaved in just the opposite manner. Gardner, on the other hand, doubts if the spleen and liver have anything to do with cholesterol formation. If it were not for the exalted position held by biochemistry in medical investigations today, such results would suggest a recommendation that technical methods be overhauled, problems be studied in fewer numbers but more intensively, and conclusions more conservatively drawn.

The peculiar lipoid cell changes in Gaucher's disease require mention, because the spleen, as a prominent locus of the reticulo-endothelial system, is intimately involved in this condition. As the essential nature of the disease is unknown, however, it can as yet throw but little light on this phase of splenic function.

d. Iron and pigment metabolism. The peculiar richness of the spleen in iron and blood pigment was noted by Henry Gray in 1854, and by others too numerous to mention since that time. The latest observers, Ziegler and Wolf (1924), found it richer in iron than any of the organs of the body in all the species examined by them.

This fact together with the close association of iron with the hemoglobin molecule and the changes observed in iron elimination after splenectomy have led Asher and his school to consider the spleen the important organ of iron metabolism. They found that splenectomized puppies excreted more iron than did controls from the same litter (Grossenbacher) and that this increase persisted even ten months after splenectomy, when compensatory processes would presumably have had a chance to operate (Zimmermann). Experiments in our own laboratories (*Spleen and Anemia*, p. 112 and 191), with a larger number of animals of nearer the same weight, using longer test periods and partly with the same method of determination (Neumann), failed to show any important changes after splenectomy and we were inclined to explain Asher's results on these grounds.

The only one of our dogs showing any noteworthy increase in iron elimination was also the only one with a marked anemia and we felt that the relationship was rather with the anemia than with any direct action of the spleen. This would be supported by the increased iron elimination in some anemias and by Schmidt's findings that in mice anemic from an iron-poor diet, the demonstrable iron disappeared from the liver much more than from the spleen. In Asher's laboratory, Vogel found that a diet rich in iron would relieve the anemia that splenectomy produced in animals fed with food free from iron (from their point of view thus compensating for the lost organ of iron metabolism); but in our hands splenectomy produced anemia even in the presence of an iron rich diet. Bayer and Roth (quoted by Asher) found an increased output of iron following splenectomy in man, whereas splenectomy for such conditions as hemolytic jaundice and pernicious anemia is followed by great reduction in the output of iron, coincident with improvement in the blood picture—obviously due to the lessening of blood destruction, however that may be brought about. I can find only one observation bearing on this point in the recent literature. Belak and Ságley found that injections of electroferrol failed to bring about a rise in the blood picture of splenectomized animals, similar to that obtained in the controls. We must leave the solution of this interesting problem for further future work. Is it possible that the spleen is needed in iron utilization from the point of view not so much of preventing its loss by excretion as of so acting upon the iron that it becomes available to the bone marrow for building up the hemoglobin molecule?

8. Relation of the spleen to other organs. *Hemolyto-poietic system.* Various relationships of the spleen to other organs have necessarily been outlined in the preceding sections, chiefly depending on the assumption after splenectomy by these organs of the spleen's lymphoid or reticulo-endothelial rôle. The possibility of interaction, however, of the spleen with other glands of internal secretion, even though poorly understood in most particulars, requires special consideration at this point. As Eppinger has emphasized in *Die Hepatolienalen Erkrankungen,* the spleen is particularly closely bound to the liver and he believes in a reciprocal relation between the two organs, which involves more than the disposition of the products of blood cell destruction.

In Asher's laboratory, too, it was found that the increased acetone formation that followed a carbohydrate-free diet was diminished in splenectomized dogs, from which it was deduced that the spleen had an

activating effect on acetone formation by the liver (Gutknecht). In the same laboratory, also, this was confirmed by Kobayashi, who showed that the perfused guinea pig liver formed acetone from sodium butyrate and that spleen extract increased this activity fourfold, though intestinal extract (which also contains cholin) failed to do so. Here, too, may be mentioned Ebnöther's observations on the hemolytic power of spleen and liver extracts. Like the majority of previous investigators (*Spleen and Anemia*, p. 78), he had found that splenic extract had scarcely any hemolytic power in vitro, and that the liver had but little more; but when the two were combined, he observed that the hemolytic activity was increased out of all proportion—all tending to show that, in some way quite unknown, the spleen has a mission of activating liver function.

Thyroid. Against the thyroid, the spleen has been thought by the Asher school to have an antagonistic action: splenectomized animals are more susceptible to oxygen lack than normal animals, thyroidectomized animals less so (Streuli); the thrombin content of the serum is diminished after splenectomy, increased after thyroidectomy (Yamada); the spleen inhibits blood formation, the thyroid stimulates (du Bois). (N. B. In the fourth section of this article I have given the reasons for believing that the spleen stimulates, not inhibits, blood formation.) The diminished susceptibility of the thyroidectomized rats is denied by Klinger, who also takes exception to Yamada's deductions. At best these observations await confirmation.

Parathyroid. A connection between the activity of the spleen and the parathyroid is suggested by the observations of Hall and Ablahadian that the intravenous injection of a splenic extract into rabbits caused a marked rise in blood calcium, greater than that obtained with their parathyroid extract. This reached its height in about twenty-four hours and returned to normal in twelve to fourteen hours, unless prolonged by the addition of parathyroid substance to the injecting fluid. Without giving figures, they state that in two splenectomized rabbits, the blood calcium dropped almost fifty per cent, but returned to normal after administration of the splenic solution. These interesting observations certainly call for further study. Unfinished work of our own tends to confirm them to a certain extent.

Thymus. Investigations of possible spleen-thymus connections have as usual produced contradictory results. Thus definite changes of several kinds in the spleen after thymectomy have been reported by Magnini, Matti, Klose, Perrier and Baggio; while Soli, Pappenheimer and Lucien and Parisot failed to observe any noteworthy changes.

Conversely Matsuno, Mann and Marine could not find that removal of the spleen produced any effect on the thymus.

Ovaries. Using the iron content of organs (as determined by the new Kugelmass micro method) as a test of the activity of iron metabolism, Tominaga found that this was not influenced by removal of the ovaries. Incidentally he confirmed the fact that the spleen is the richest of all organs in iron and found that after splenectomy the liver and kidney held more and the muscles less iron.

Pancreas. Special relations between spleen and pancreas have been contemplated for centuries (Daniel Duncan in 1683 thought that the pancreas was an "emunctory for the acid humors" formed in the spleen); but have been more actively investigated since Schiff's studies suggested that the spleen elaborated a *quid necessarium* for the formation of trypsin. The preceding section, however, shows that the great weight of evidence is against any such hypothesis. A possible synergistic relation between the spleen and the pancreas of unknown nature, is suggested by the work of Sweet and Ellis. Studying the effects of destruction of the "external function" of the pancreas by removing most of the organ, they noticed that the spleens of the test dogs were constantly reduced in size and weight to a degree several times greater than that of the other organs or the general body weight. Inlow confirmed this peculiar behavior of the spleen, but as it was duplicated in ordinary starvation, considers it due to the inanition resulting from the exclusion of pancreatic juice from the intestine rather than a specific spleen-pancreas relationship. Neither author attempts to explain the reason for the extraordinary atrophy of the spleen, which must at least in part be due to and made more apparent by the uttermost use of its reservoir function.

GENERAL CONCLUSIONS

The mammalian spleen, while not necessary for the maintenance of normal existence and sharing many of its functions with other members of the hemolyto-poietic system, is useful in several ways and an organ whose presence under certain stresses may even be the deciding factor between life and death. Its functions are largely indicated by its structure, its reticulo-endothelial cell content and by the changes produced in other organs by its absence.

There is good reason to consider it a blood reservoir that can be called upon efficiently to meet various physiological and pathological demands.

It is directly concerned in blood cell formation during fetal life and can revert to this function upon demand at any period of existence. It continues to furnish lymphocytes, large mononuclears and possibly other blood cells through most of adult life and has an indirect influence on blood formation, through a stimulating action on the bone marrow, possibly after activation by the liver.

It is intimately concerned in the processes of red blood cell destruction, a rôle greatly increased in certain pathological conditions. It not only, in some way as yet unknown, renders circulating erythrocytes more fragile, but through its reticulo-endothelial cells, has the ability to scavenge blood cells and bacteria from the blood. It takes up disintegrating erythrocytes as a step in blood pigment metabolism, either in the form of fragmented cells, hemoglobin bearing dust or even, in conditions of increased hemolysis, of whole cells (graveyard function). It not only is the richest hoarder of iron in the body, but also prepares bilirubin pigment from the broken down hemoglobin for passage to the liver. In the absence of the spleen it becomes more difficult to produce jaundice with hemolytic agents. Continental authorities consider it the controlling organ of iron metabolism in the body.

It seems to be an important site of antibody formation (again through the reticulo-endothelial cells), though this function is quickly taken up by other organs in its absence. Through its lymphoid rôle, it also plays a part in resistance to such infections as tuberculosis. Preponderating evidence points to its also being concerned with biological resistance to tumor growth.

Its relation to metabolism is less manifest. Disturbances in growth and digestion following its removal, maintained by some, are denied by more; and the same can be said of its relation to basal, nitrogen and carbohydrate metabolism. It seems to have a more definite connection with fat metabolism, especially cholesterol and the unsaturated fatty acids, which may be the reason for the improvement which splenectomy often procures in clinical anemias, though here too authorities are far from unanimous.

With regard to its relations to other organs, the liver, lymph nodes (lymphoid tissue generally) and bone marrow, as members of the hemolyto-poietic system, are so closely allied that they share certain of its functions normally and quickly take over the remaining share after extirpation of the spleen. It has special affiliations with the liver on account of its definite "upstream" position in the portal circulation and possibly others of a more specific incretory nature. An

interesting association with the parathyroids in calcium metabolism has recently been suggested. Supposed antagonistic relations with the thyroid await confirmation; connection with the thymus or ovary apparently does not exist and a possible synergistic relation to the pancreas is probably better explained as an especially marked participation (perhaps through its reservoir function) in the effects of inanition, rather than by a specific hormonal connection.

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